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CHROMOSOME STRUCTURE IN RELATION TO THE CHROMOSOME CYCLE. II[1]

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INTRODUCTION

The discovery that chromosomes divide longitudinally, and that the halves so produced separate into two nuclei in the cycle of mitosis, represented a fundamental step in the analysis of the processes which living cells effect their reduplication. A more precise definition in terms of chromosomal materials followed the disclosure that the chromonema is not a transitory component of the chromosome but that it maintains the linear order of the genes through successive mitosis. In the cycle of coiling and uncoiling attendant on multiplication, the chromonema reflects on a microscopically detectable level the adjustments requisite to duplication of the constituent genes and their distribution to daughter nuclei. Submicroscopic alterations that occur in the form and substance of the chromosomes during the mitotic processes represent a further level of analysis, toward which an approach has been made by application of physical and chemical methods developed for studies of cellular constituents. The contributions that have been made in recent years toward an understanding of these phenomena form the basis of the present report. This serves as a supplement to a previous review (185), which outlined the more general features of the chromonema cycle and appraised the divergent interpretations of such particulars as number, linear organization, mechanism of coiling, and relation of the chromonemata to other materials of the chromosome. Since the 1936 review was written, a series of other summaries, critical reviews and expressions of opinion on these controversial matters has been presented (87, 92, 132, 133, 140, 143, 169–171, 209, 273, 283, 294, 295, 320, 356, 358, 360, 383, 403, 404, 427, 430, 431). Because of this extensive series the review offered here makes no pretense at completeness in surveying the literature that has accumulated during the past ten years but aims rather to indicate the present level of our knowledge of chromosome structure and prospective approaches to further studies.

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A considerable portion of the information now available concerning the organization of the chromosome during somatic and meiotic mitoses has been obtained from studies of such plants as *Tradescantia* and *Trillium,* although in recent years a number of supplementary observations of the behavior of chromonemata have been made on several species of animals belonging to various phyla (*e.g.*, 72, 78, 80, 135, 193, 199, 235, 265, 287, 302, 315, 332, 414, 428, 432). Studies on giant chromosomes, such as those of the salivary glands of Diptera and the oocytes of Amphibia, have been of paramount importance in resolving conflicting viewpoints. Whereas the larger plant chromosomes provided favorable material for basic studies of the pattern of coiling of the chromonemata, the giant chromosomes have been indispensable in the more recent histochemical approaches to problems of fundamental organization.

Newer methods of analysis developed in these studies have involved the use of monochromatic ultraviolet radiation for identification and determination of the distribution of cellular components during the cycle of mitosis. The technique permits measurements to be made of amounts of substances that could not be detected by other chemical or physico-chemical methods (51). Different parts of the spectrum and different ranges of absorption require different procedures. A photoelectric method with electrometers as recording instruments, which is applicable to objects of a diameter of 1 μ or larger, has been designed for use with ultraviolet radiation beginning at 2,000 Å, as well as with the visible portion of the spectrum. For analysis of finer detail, the method of measuring density differences on photographic plates with a recording microphotometer is applicable within the limits of the resolving power of the microscope lenses. (Descriptions of these methods of analysis, and illustrations of the apparatus used, are to be found in papers 47, 51 and 74.)

Other advances in methods of analysis include the development of the phase-contrast and electron microscopes. In the phase-contrast system, slight invisible phase changes in the light passing through the specimen are converted into intensity differences that can be seen (the physical principles involved are discussed in 18, 38, 197, 329 and 441). As a result, a wealth of detail may be disclosed in cells that appear relatively homogeneous under the ordinary microscope (see 17, 18, 229, 264, 327–329 for a discussion of the general adaptability for cytological studies). Since chromosomes may be identified in the living or unstained cell, phase-difference microscopy gives promise of serving as a useful supplementary method in experimental approaches to problems of chromosome structure.

The electron microscope, on the other hand, provides a manifold extension of the power of resolution beyond that available with optical systems using ordinary light, and should ultimately prove serviceable in the analysis of the finer details of chromosome organization. Sharp definition, combined with great depth of field, is attainable at magnifications as high as 20,000 diameters (442). However, a vacuum is required throughout the instrument to permit passage of the electrons, which have low power of penetration; and for the same reason only extremely thin layers of thoroughly dehydrated material can be analyzed. These requirements constitute severe handicaps in the study of biological materials. Some progress has been made in analyzing the structure of dispersed particles of small dimensions, such as viruses, bacteriophages (see review in

443) and isolated mitochondria (69), and in determining the organization of the cytoplasmic ground substance and inclusions in smears (326) and outgrowths of explants of embryonic cells (309). Such methods have but limited application for the study of chromosome structure (*cf.* 68), and sectioning must be employed if normal spatial relationships are to be maintained. Several types of microtome, each of which has certain limitations, have accordingly been designated to permit preparation of sections as thin as 0.1 μ (4, 5, 129, 145, 289, 324, 325). In addition to the difficulties of sectioning, special problems arise in efforts to insure faithful preservation of structural details during the processes of fixing, embedding and dehydrating (70, 115, 116, 145, 440). It is thus apparent that the technology of electron microscopy is at present in the preliminary stages of development with respect to its utilization for cytological studies; but if new methods are developed that will permit examination of thin slices of embedded cellular material, the resolution of finer details of chromosome structure may be anticipated. The need for such information will be apparent as the conflicting viewpoints arising from different interpretations of the same type of material are presented in the following pages.

ORGANIZATION OF THE CHROMOSOME

The compact, cylindrical chromosome, such as is seen at anaphase of mitosis, contains within its limiting membrane (sheath or pellicle) the helically coiled chromonemata and the associated matrix. A primary constriction marks the position of the centromere (spindle attachment region or kinetochore). Secondary constrictions are often detectable, such as those that separate terminal satellites and reveal the positions at which the nucleoli are organized during the telophase stages. Other secondary constrictions may exist at various positions along the chromosome, and are usually seen most clearly during late prophase stages. Utilizing the constrictions and length of arms as topographical markers, the karyotypes of many species of plants have been described in recent years. These studies need not be reviewed here, especially since many of them have been concerned primarily with phylogenetic relationships rather than with problems of chromosome structure. Moreover, a comprehensive index to the literature dealing with chromosome numbers in plants has recently been published (94).

Sheath

The existence of a membrane which delimits the condensed chromosome has been inferred from various aspects of chromosomal behavior in normal and experimental material (*e.g.*, 261, 262, 356). In microsporocytes that have been partially flattened by spreading beneath a scalpel on a glass slide prior to fixation, the coiled chromonemata are surrounded by a material differing in its microscopic appearance and staining capacity from the cytoplasm, and presumably separated from it by a delicate interfacial membrane. The aspect in a general way resembles that of the karyomeres that are formed at telophase in some grasshoppers, fishes and other animals. Prefixation treatment—as with KCN—of the condensed chromosome of metaphase or anaphase will produce a similar appearance (176). Demonstration of a chromosomal membrane in the living cell is extremely difficult, but has been obtained by application of micrurgical methods to the salivary-gland chromosomes of the larva of *Chironomus* (82), confirming the observations made by other workers on fixed and stained preparations of these giant chromosomes (*e.g.*, 196, 358).

In some species of animals more conspicuous perichromosomal coatings have been observed. The sheath of the metaphase chromosomes in some Hemiptera appears to be modified to form interzonal connections between the separating daughter chromosomes at anaphase (352, 353). Treatment with osmic acid will blacken a broad zone of material surrounding the chromosome in spermatocytes of the beetle *Palomena* (164). Confluence of perichromosomal sheaths under special conditions may lead to formation of chromosome aggregates (262, *cf.* 357).

From this limited description it is evident that the sheathing materials vary greatly in their morphological aspects in different cells. Further information concerning the chemical nature of the materials of the pellicle, and their relation to other chromosomal and cytoplasmic materials, is to be sought in the application of histochemical methods such as are described subsequently.

Matrix

Although it is generally agreed that the condensed chromosome is bounded by a sheath of Feulgen-negative material, there is no unanimity of opinion concerning either the nature of the matrix material or its distribution within the chromosome. The term "matrix" has been used in a variety of ways, some authors employing it as a synonym for sheath or pellicle, others applying it to that portion of the chromosome, seen in fixed preparations, that envelops the chromonemata. On the other hand, some workers (especially 84 and 96), although admitting the existence of a chromosome pellicle, have maintained that the matrix of fixed material has no relevance to the living chromosome. This point of view could be supported more readily if it were assumed that the material of the so-called matrix seen in stained preparations represents the Feulgen-positive coating of the individual chromonema, and if there were no additional material lying between the chromonemata and the chromosomal membrane. With respect to the first of these qualifications, available evidence suggests that the desoxyribose nucleic acid is not a superficial deposit on the genonema, in the sense of a loosely-associated or enveloping matrix substance, but that it is bound up with the proteins of the thread itself. The second qualification has been critized repeatedly (*e.g.*, 242). Although in some chromosomes the chromonematic coils are packed so tightly that they seem to constitute a closed cylinder (101, 404, 428), it has been demonstrated, both in living material (66) and in fixed preparations, that the end view of a chromosome often presents the aspect of a hollow cylinder, with the more refractive or densely staining material peripherally disposed (*e.g.*, 415, 391, 144). Additional and more striking evidence is provided by those preparations in which, as a result of prefixation treatment, both pellicle and chromonemata have been separated to reveal an intervening zone—the matrix—as has been shown in photographs of *Trillium* (176), of *Tradescantia* (408, 412), and of the grasshopper *Podisma* (235). Since a different interpretation of this type of fixation image has recently been proposed (332), it is necessary to consider its validity insofar as it bears on the problem of interpretation of interchromonematic materials. Ris suggests that Makino's photographs of *Podisma* indicate that four chromonemata are present in each of the homologues, and that two of the strands are peripherally disposed and stain faintly, the other two remaining appressed in the medial region and staining intensely. This interpretation leaves unanswered a series of questions concerning the disposition of sister chromatids and half-chromatids in the axial and peripheral regions of the chromosome, and their capacity for differential staining. Moreover, the appraisal need not be based exclusively on orthopteran material, since the same general aspect of a bivalent that is shown in Makino's photographs was also presented by those preparations of *Tradescantia* in which the four chromatids of first meiotic metaphase were first demonstrated to be helically coiled threads (182, figs. 62 and 63). There seems little question, in the light of the considerable number of studies on the chromosomes of this plant in the intervening years (see especially 208), that the two chromatids constituting the dyad are intimately associated, and that, although each may be further subdivided (to provide an octopartite bivalent, as will be described subsequently), the half-chromatids are closely appressed and not loosely associated in the two-by-two relationship assumed by Ris to exist in the grasshopper chromosome.

The material constituting the matrix in these *Tradescantia* preparations does not stain with gentian violet-iodine, aceto orcein or the leucobasic fuchsin used in the Feulgen technique for detection of desoxyribonucleic acid; accordingly, it has often been designated as achromatic. From the evidence obtained by studies of salivary-gland chromosomes it seems that the interchromonematic material contains a pepsin-digestible protein, since chromosomes exposed to the action of this enzyme undergo a marked reduction in diameter (127, 128, 257, 258, 365). A similar but less pronounced effect of pepsin on the root-tip chromosomes of the onion has been reported (reference in 257). Removal of the interchromonematic protein does not disrupt the "skeletal" proteins to cause structural disorganization of these chromosomes, nor is the Feulgen-staining capacity of the desoxyribose nucleic acid impaired by pepsin digestion. It has also been reported (358) that the interbands of the salivary-gland chromosomes of *Drosophila,* which are Feulgen-negative in contrast with the Feulgen-positive bands, can be counter-stained with fast green, using the method employed for the differential staining of nucleoli (24–26, 367–369). Similarity in the staining quality of matrix and nucleolus is not in itself unequivocal evidence in support of the contention—previously suggested on morphological and cytogenetic grounds—that they contain similar materials. However, the enzyme ribonuclease will remove the fast-green staining component from the chromosome (358). It appears, therefore, that ribonucleoproteins, which are known to exist in the nucleolus

(363), also represent a major component of the matrix (*cf.* 32). However, the conclusions that may be derived from these tests are limited by questions concerning the purity of the enzyme and its specificity of action. Some samples of crystalline ribonuclease tested have indeed been found to possess proteolytic activity (73, 349).

Both the ribose and the desoxyribose nucleic acids show maximum absorption in ultraviolet radiation of about wave length 2,600 Å. When chromosomes of the pollen tubes of *Tradescantia* were exposed to radiation of wave length 2,537 Å, they were markedly shortened and disclosed a hyaline matrical zone. It was suggested that this effect might be attributable to absorption of the ultraviolet radiation by ribonucleic acid of the matrix (408). Chromosomes may be broken as a result of exposure to ultraviolet rays as well as X-rays, although there are marked qualitative differences in the effects of the two kinds of radiation. The possible rôle of the matrix in the process of chromosome breakage was outlined briefly in the original (1936) review. More recently it has been suggested that the difference in the types of alteration produced by X-rays and by ultraviolet rays may be due to the fact that the latter can not disrupt the matrix to produce a thoroughgoing break, whereas the X-ray break may involve both chromonema and matrix (272). The inhibition of X-ray-induced breaks in *Tradescantia* by ultraviolet radiation of wave length 2,537 Å has also been attributed to the effect of the supplementary treatment on the substance of the matrix (413).

In contrast with the point of view that the matrix material is essentially achromatic, a number of cytologists in recent years have accepted the interpretation that the matrix represents the Feulgen-positive coating of the gene string or genonema (*e.g.*, 140, 160, 356, 371). Designation of this portion of the chromosome as the matrix may be referable to a series of factors—among them the gradual accretion of thymonucleoproteins by the genonemata as the chromosome increases in diameter during the prophases; the aspect of uniformly colored, Feulgen-positive metaphase and anaphase chromosomes when fixation does not disclose the details of internal organization; the presence of chromatic interchromosomal connections, even in living cells of some species; and the assumption that the material of the matrix is indispensable in the processes of gene multiplication and metabolism and must therefore contain thymonucleic acid as an essential component. Thus the connotation of the term "matrix" has undergone considerable revision in the course of recent years (see 283 for various interpretations). A more rigid definition seems required if confusion is to be avoided in describing the findings of the increasing number of cytochemical studies of chromosomes (*e.g.*, 121). Much remains to be determined about the synthesis and distribution of materials within the chromosome; and little precise information can be offered at present concerning the relationship of the matrix to the chromonemata during the various phases of mitosis, although an elaborate scheme has been proposed relating the rôle of the matrix in the spiralization cycle to changes in its colloidal state (209). Despite these limitations, the results available from physical and chemical methods of analysis, together with the older morphological considerations, lead to the suggestion that the term "matrix" should be reserved for the portion of the chromosome surrounding the chromonemata, which can be dissociated from them by various techniques. The term "chromonema" may continue to serve as a morphological description of the genonema and its associated desoxyribose nucleoproteins. With an increasing knowledge of the chemical nature of chromosomes, a terminology based on morphological considerations may undergo drastic revision; until such precise information is available there seems little advantage in applying new terms to describe the Feulgen-positive material around the genonema.

Centromere

Before turning to a consideration of the number of chromonemata and the patterns of their coiling, attention will be given to various aspects of the problem of linear organization of the chromosome. The centric region is a primary topographical feature. It occupies a definite position with respect to the ends of the chromosome that may be altered by such processes as pericentric inversion, duplication, unequal reciprocal translocation or misdivision of the centromere. Compound chromosomes, as occur in the germ line of *Ascaris megalocephala,* presumably contain the centromeres of the several component units (300, 353, 426). A type of centromere that is not localized but spread over the chromosome occurs in the Hemiptera (166–168, 331, 352, 353, 418). When chromosomes of *Steatococcus* and *Tamalia,* having such diffuse centromeres, were fragmented by X-rays, each of the segments maintained the normal centric functions; it continued to divide through successive mitoses and did not behave as an acentric body.

It is generally assumed that the centromere represents a compound structure, although the distribution of its components has been the subject of much speculation. Various considerations indicate that the chromonemata, perhaps in a modfied form, traverse the body of the centromere (*e.g.,* 253). Such structural organization would best explain the free separation in successive stages of the numerous chromonemata that constitute diplo- and polychromosomes (10, 151, 152). Following differential staining, small spherical bodies (spindle spherules or attachment chromomeres) have been observed within the centromere (*e.g.,* 355), presumably at the point of union of the chromonemata extending in from the two arms of the chromosome. At the time of initiation of the anaphasic movements, the spherules may be drawn out but maintain their connection with the body of the chromosome by attenuation of the chromonematic threads. Counts of the number of detectable attachment chromomeres suggest that one is present for each functional chromatid (*e.g.,* 177, 354). Thus in *Trillium* and in *Lilium* at first meiotic metaphase there are six granules detectable in a trivalent, four in a bivalent, and two in a univalent (177). The observation that two granules are present in the metaphase chromosome of the second meiotic division is regarded as evidence consistent with the interpretation that the chromosomes during the first meiotic division are octopartite. In some plants the fine threads and the attachment chromomeres were found to be Feulgen-positive (177, 312); in others the material of the centromere gave a negative Feulgen reaction (343, 344). Such dissimilarities may possibly be attributable to difficulties in interpreting the Feulgen reaction when applied to bodies of minute size, and not to fundamental chemical differences between the materials tested (*cf.* 356).

Modification of chromosome form in the course of mitosis—for example, the production of an isochromosome with a median centromere and two structurally similar arms from a chromosome with arms of unequal length—has been observed in a number of plant genera, *e.g., Tulipa* (422), *Pisum* (198), *Fritillaria* (88, 90), *Zea*

(322), the B-type chromosomes of *Sorghum* (100), *Nicandra* (93), *Secale* (219, 275–277), *Nothoscordum* (130) and *Godetia* (155, 156). The origin of such modified types has been attributed to misdivision of the centromere (90). A pattern of organization of the centromere which would permit such misdivision has accordingly been visualized (88, 283). The essential organ of division in this pattern is a centric fluid, normally "exploding", according to Darlington, so as to divide the centromere parallel to the long axis of the chromosome in accordance with the distribution of the fibrous determinants or centrogenes, but capable of division in other planes under special conditions. On the other hand, the possibility of origin of isochromosomes from abnormal attachment of chromatid segments to the halves of a normally divided centromere has been advanced (277). Metacentric chromosomes, morphologically indistinguishable from isochromosomes, apparently originate from J-shaped chromosomes during meiosis in *Gasteria* as a result of crossing-over in a heterozygous pericentric inversion (148).

When a chromosome with a truly terminal centromere (a telocentric or telomitic chromosome) originates, it apparently either is eliminated in natural selection or gives rise to an isochromosome. The behavior of a telocentric chromosome in maize indicates that it regularly undergoes structural changes in somatic cells (322). Such instability may apply to all such chromosomes and account for the fact that they are rarely, if ever, found in nature. Even such small chromosomes as the dot-like fourth of *Drosophila melanogaster* are two-armed, as cytological and genetical evidence has shown. The confirmation in recent years by several workers (78, 81, 86, 265) of White's earlier observation that the chromosomes of grasshoppers possess minute short as well as long arms appears to invalidate the descriptions of truly telomitic chromosomes in these insects (e.g., 161). However, the pronounced short arm shown in Coleman's photographs of chromosomes of *Chortophaga viridifasciata* (78) is not indicated in Carlson's illustrations of this material (43, 44).

Division of the body of the centromere occurs with the onset of anaphase. As the daughter chromosomes move apart the centromere takes precedence, the attachment chromomere marking the position of the spindle fiber that extends from the chromosomes to the pole. Exceptional cases have been reported of chromosomes having secondary centric regions that lead the way to the poles in meiotic divisions, sometimes even before the primary constriction has divided (323, in maize; 181 and 310, in some inbred strains of rye). A further analysis of these actively mobile, so-called T-ends of the rye chromosome in meiosis indicates that they are furnished with chromosomal fibers similar to those attached to the centromere (292). The possibility of a shift in position of a functioning centromere within a chromosome had been suggested previously by Carothers (45), although her theory that the centromere is merely a temporarily modified chromomere is not supported by the evidence presented in the preceding paragraphs, nor by the discovery in certain viviparid molluscs of an intimate centromere-centrosome relationship (306).

Nucleolus-Organizing Region

The nucleolus is ordinarily formed at the telophase of mitosis by the nucleolus-organizer, which occupies a specific position in one of the chromosomes of the set. As the nucleolus enlarges it may displace parts of the chromosome—such as the satellite and the main body—which maintain continuity, nevertheless, by extension of the connecting chromonematic threads. During the succeeding prophases, as the nucleolus diminishes in volume, the separated parts are again brought into contact, although the position formerly occupied by the nucleolus is often revealed at late prophase or at metaphase by a marked constriction (a so-called secondary constriction). Its width, or the length of the satellite thread, at these stages, however, does not necessarily bear a direct relation to the size of the nucleolus at telophase (317, 319, 320).

Modifications of this standard pattern have frequently been reported. Duplication of the region containing the organizer may permit the development of nucleoli in additional chromosomes of the set. A striking example is provided by the male flies of *Drosophila ananassae* (186), in which the fourth chromosomes and the Y-chromosome are concerned with nucleolus formation. In the females only the fourth chromosomes carry the organizers. Since in females of related species of *Drosophila,* nucleoli are organized in the X-chromosomes, it seems probable that interchromosomal rearrangement in the course of phylogeny has resulted in the transfer of the organizer from the X to the fourth chromosomes. Duplication within the chromosome set of the segment carrying the organizer has likewise been suggested as an explanation of the extra nucleolar chromosomes of *Rhoeo* (27).

In several species of *Trillium,* in some lines of *Pisum,* in *Allium amplectans* and in *Paris polyphylla* the nucleoli, sometimes several in number, are produced at the ends of the chromosomes (250, 96, 218, 158). However, in the related species *Paris hexaphylla* and *Medeola virginiana* the nucleoli are organized at definite positions in specific chromosomes (250, 400). Such differences between closely related species could be attributed either to the absence or presence of the organizer or to relative degrees of its activity. It has been suggested that in *Trillium* the organizer is probably not absent, but of small size (316, 400). The presence of nucleoli on several chromosomes of the set, as in the somatic and pollen mitoses of *Allium amplectans,* may seem to indicate that a definitive organizer is lacking (218), although the association in this species of a single nucleolus with one pair of homologues during pachytene and diplotene stages of meiosis suggests that an organizer exists whose capacity to function varies in different tissues.

Another modification affects the timing of the cycle of organization and dissolution of the nucleoli, so that they may appear precociously at anaphase, or persist until metaphase (123, 158, 220, 260; other references in 87, p. 306). Both the precocious and the persistent types have been observed in somatic cells of *Bellevalia hackeli* (220). Satellited chromosomes, which presumably carry the organizers, have been observed in this species and also in *Pisum* (158). The nucleoli, however, were not localized in the region of the satellite, but either were budded off from the ends of the chromosomes or appeared in regions close to the centromeres. Disturbances in the normal genotypic control may thus be assumed to upset the functional capacity of the organizer, which loses its competitive advantages to other portions of the chromosomes in the organization and timing of the process of nucleolus formation (*cf.* 250). Studies of the nucleolus of several species of *Salix* indicate that persisting nucleoli are more frequent at lower temperatures than at higher ones (114). These observations have led to an elaboration of the concept of the nucleolus as a coacervate—a separated phase out of a saturated solution. Differences in the activity of the region that includes the organizer—perhaps as a result of alterations in cellular environment during phylogeny—are also shown by observations that at corresponding positions on the long chromosome in three species of *Chironomus* (*C. tentans, C. lobiferus,* and *C. plumosus*) there may occur either a nucleolus, a nucleolus-like "puff", or a dark band rich in nucleic acid (259).

It has been demonstrated in several species of animals and in some plants that the organizers occur in parts of the chromosome that are heterochromatic. During pachytene in *Zea* (232) and in *Medeola* (400), for example, the nucleolus-organizing regions are detectable as heteropyknotic knobs. In some plants, such as *Narcissus* (118), *Uvularia* (140) and members of the Aloinae (316), the amount of detectable heterochromatin is either negligible or confined to a small satellite, although the process of nucleolus formation is not impaired thereby. The relation of size of the heterochromatic region to capacity for nucleolus formation is not always a direct one; although a quantitative correspondence was found in *Solanum* (217), in *Zea mays* the smaller portion of a fragmented organizer produced the larger nucleolus (232). It has been reported that in *Narcissus* both euchromatic and heterochromatic supernumerary chromosomes effect an increase in volume of nucleus and nucleolus as compared with plants lacking such chromosomes, but that the essentially euchromatic chromosome exerts its effect primarily on the nucleus, whereas the heterochromatic supernumerary exerts a much stronger effect on the nucleolus (121).

These heterochromatic regions, which have the capacity to form large amounts of thymonucleoproteins within the chromosomes of which they are a part, appear therefore to be concerned also with the ribonucleoprotein metabolism of the nucleolus (60, 362, 363). Caspersson (54) has suggested that the essential function of the heterochromatic regions is the production of histones, which accumulate in the nucleolus during the telophase and interphase and then diffuse through the nuclear membrane to form in the cytoplasm the ribose nucleic acids that are concerned with the synthesis of cytoplasmic proteins. The method used in reaching an estimate of high histone content, on which these conclusions are based, involves interpretation of curves of absorption spectra; its validity has been questioned (268). More recently it has been reported in a brief abstract that, following application of the Millon test for protein, the ultraviolet absorption peak of tyrosine-containing proteins such as the histones is shifted away from the region (*ca.* 2,800 Å) which is largely dominated by strong absorption of the purine and pyrimidine bases characteristic of nucleic acids, into a region (*ca.* 3,500 Å) where the absorption due to nucleic acid is negligible. Using this criterion for detecting the tyrosine content before and after removal of histone, it has been found that in the nucleolus of maize histone does not constitute more than 10 per cent of the total protein (307). More data will be required before such results can be appraised adequately with respect to Caspersson's hypothesis, especially since earlier work had shown that different types of nucleoli may vary with respect to the proportions of protein and nucleic acid (363). Caspersson's conclusion has been questioned, however, on the basis of observations that supernumerary euchromatic as well as heterochromatic chromosomes exert an effect on the volume of the nucleolus in *Narcissus* (121, see also 320). These observations suggest that the nucleolus is derived from materials provided by all the chromosomes at telophase, the heterochromatic sections being more efficient than the euchromatic in nucleolus formation. This represents essentially a reaffirmation—in terms of chromosomal constituents—of the transportation hypothesis visualized by some earlier workers from morphological considerations of the chromosome-nucleolus relationship.

Other Secondary Constrictions

Secondary constrictions other than those associated with nucleolus formation have been observed in the chromosomes of many plants and animals. They are generally most conspicuous during the prophases, and may be lost to view in the condensed chromosome (*e.g.*, 405). A striking illustration of a secondary constriction is provided in the left limb of the second chromosome of *Drosophila melanogaster,* the adjacent parts of the chromosome sometimes being so widely separated during the early prophases as to appear as separate chromosomes, although critical observation reveals that an attenuated chromonematic thread bridges the intervening gap (184).

It has been suggested that secondary constrictions, other than those attributable to the activity of nucleolus organizers, represent intercalary heterochromatic regions. This generalization rests on the discovery that exposure to low temperature will produce in the chromosomes of *Trillium* short terminal or intercalary segments, having so little thymonucleic acid that they present the appearance of a series of secondary constrictions (95–97). A similar annulated appearance has been produced in the chromosomes of a series of plants (142, 179, 180, 219, 321, 433) and in the salamander, *Triton* (39, 432), by exposure to cold. Factors other than cold treatment may be effective in disclosing these "differential segments" in chromosomes that do not reveal them under normal conditions. They may be induced by starvation of animals (432); and they have appeared in hybrids of *Mecosthetus* (194). In haploids of rye "nucleic acid starvation" seems to occur under normal conditions (219, see also 320). Intercalary segments with a staining reaction different from that of adjoining regions have been revealed in some root-tip chromosomes merely by the application of special methods of fixing and staining (203, 222).

It has been reported that the differential segments are constant in number, although they may vary in length (96, 433, 434), and that they occupy specific positions along the chromosomes. Differences in homologous chromosomes of *Trillium* have been attributed to the hybrid nature of the material (96), although other workers have found that in plants of this genus a chromosome may be regionally differentiated in some cells of a single plant and not in others. Considerable variability in the expression of "negative heterochromacy" has also been reported to occur in different cysts of a single testis of the grasshoppers, *Mecosthetus* and *Chorthippus* (194). Similar variations have been reported among the chromosomes of different nuclei of an individual in the Salmonidae (405). The possibility of utilizing patterns of distribution of differential segments for detecting variations in chromosomal organization may accordingly prove useful in some species but not in others.

Darlington and LaCour have suggested that the differential segments represent regions in which the accumulation of nucleic acid that occurs under normal conditions has been inhibited. In support of this interpretation they have reported that there is a positive correlation between the number of differential segments and the number of "chromocenters" detectable in the resting stage. These deeply staining bodies have long been recognized as heterochromatic portions that differ from other parts of the chromosome in the timing of the nucleic acid attachment in relation to the cycle of reproduction ("Heterochromatin is allocyclic."). It is also known that the chromocenters exhibit a constancy in form, number and position that is characteristic for the individual or species (*e.g.*, 109, 260, 270, 313, 316, 320). Darlington and LaCour conclude, therefore, that the over-nucleated chromocenters of the resting stage represent the under-nucleated differential segments of metaphase. In haploid rye the differential segments and the heteropyknotic knobs seen at pachytene occupy corresponding positions in the chromosomes (219). The possibility has not been excluded, however, that the regions which presumably show "nucleic acid starvation" represent exaggerated secondary constrictions, such as occur in *Drosophila* between the proximal heterochromatic and the more distal essentially euchromatic regions (the pronounced constriction in the left limb of the second chromosome of *D. melanogaster* mentioned previously provides a good illustration). This type of organization would also provide a positive correlation between chromocenters and "differential segments". As has been pointed out (359), the "starvation" interpretation is difficult to reconcile with the opinion expressed by Darlington (92) that heterochromatic regions are centers of nucleic acid synthesis. It must also be kept in mind that differential staining may be referable primarily to a difference in contraction of various parts of the chromosome, which is a manifestation of differential coiling (434; however, *cf.* 319, 321).

A comparative study of several species of *Paris* suggested that the same genes that control allocycly in one species may not do so in another. This control has been related to the activity of the nucleolus organizer, and the suggestion offered that the "absence of allocyclic behavior goes with the presence of nucleolus organizers" (91). The validity of the cytological observations on which this suggestion was based has been questioned (320); and allocycly and organizers have been reported to exist together in several species of plants and animals (39, 143, 419, 432).

Confirmation or refutation of the generalization that all secondary constrictions reveal the location of heterochromatic regions must await the accumulation of information concerning the organization of specific chromosomes. Present knowledge of the distribution of euchromatic and heterochromatic segments, even in such a carefully studied chromosome as the X of *D. melanogaster,* is far from complete. From the data now available (187, 192, 311) it is evident that some of the intercalary heterochromatic regions of the X-chromosome are not revealed as constrictions in the ordinary fixed and stained preparations of mitotic prophases. An additional indication that secondary constrictions may exist apart from heterochromatic regions is provided by the observation that in neuroblast chromosomes of stocks carrying translocations a constriction frequently marks the point of attachment between the non-homologous chromosomes (*e.g.*, 105). Since the induced breaks involved in rearrangement have been found to be distributed at random along the chromosomes, and not restricted to heterochromatin, it appears unlikely that the sample of translocations in which the constrictions were observed would include only exchanges involving heterochromatin. Chromosomal rearrangement occurs frequently in nature, and some of the constrictions that now characterize specific chromosomes, as in *D. melanogaster,* may have arisen in this process.

Chromomeres

A consideration of the linear differentiation of the chromosome must inevitably face the problem of the nature of the chromomere-like regions seen along the extended chromonemata during the prophases of somatic and meiotic mitoses. These regions frequently have been shown to be so constant in number and form that their pattern of distribution serves to identify specific chromosomes (*e.g.*, the studies of the chromomeres during meiotic prophases in liliaceous plants, 15, 16, 159, 172; those on the chromosomes of *Zea mays* and related genera, 223–227, 230; on *Crepis* and *Trillium,* 203, 204; on grasshoppers, 42; on the pachytene pattern in human spermatocytes, 361; and many others). The term "chromomere" has thus received a connotation that in modern cytological usage implies the existence of constantly reproducible regions that are set off from the adjoining portions of the chromonema by differences in diameter and staining capacity. Alexander and Bridges (3) suggested several years ago that the chromomeres seen at prophase are local accumulations of freshly synthesized materials, and serve as an index of the relative activity of the synthetic and accumulative processes carried out during these stages at different loci. In more modern terminology the chromomeres are chemically identifiable by specificity in the cycle of attachment and detachment of nucleic acid (96, 99, 204); or, as stated by Pontecorvo (308), "each chromomere shows a distinct reactivity of its own in nucleic acid synthesis; it is 'allocyclic' in relation to others, either because its cycle is shifted in time, relative to that of other chromomeres, or because different chromomeres synthesize at different rates or reach different final charges".

Interpretations that involve regional structural differences in the chromonema due to differences in the time of attachment of nucleic acid have been questioned from time to time, most recently by Ris (332), who attributes the observed linear differentiation to modifications in the degree and pattern of coiling of a thread of essentially uniform diameter. Certainly some of the chromomere-like bodies can be resolved into differentially coiled regions under proper conditions of fixation and staining. The condensed regions lying adjacent to the centromeres have been shown in some plants to represent precociously coiled threads (*e.g.,* in *Velthemia* and *Rhoeo,* 76, 77); it has also been demonstrated that the chromocenters of a series of plants and animals can be transformed experimentally into a loose mass of uncoiled chromonemata (215, 381). The chromonemata of the heteropyknotic regions that lie adjacent to the centromeres in the chromosomes of *D. melanogaster* are coiled during the prophases of somatic mitoses (184), but it has not been determined by cytological observation whether the chromomeres that appear along these chromosomes—which presumably represent intercalary heterochromatic regions—likewise represent coiled portions of the thread. On the basis of present knowledge of the organization of somatic and meiotic chromosomes, it seems certain that helically disposed chromonemata must constitute the chromomere-like segments that can be produced in some condensed chromosomes by pretreatment or by fixation which accentuates the intervening secondary constrictions (*e.g.,* 136, 179, 203, 382, 406). Even in well-extended chromosomes, such as those of the leptotene and zygotene stages of meiosis, the small chromomeric bodies have been identified as tight coils (28, 183, 201, 202, 279, 412).

The conflict between the interpretation advocated by Ris and the more widely accepted view of the reality of ultimate chromomeres, as visualized by Belling, is not to be resolved, however, by showing that some chromomere-like bodies are coiled structures, but rather by the demonstration that all chromomeres represent differentially coiled parts of the thread. Any analysis directed toward this end must consider the organization of giant chromosomes such as are seen in the salivary glands of the larvae of the Diptera. The appearance of discs or bands that traverse these so-called polytene chromosomes depends, according to the most widely accepted interpretation, on the close approximation or confluence of homologous chromomeres of a series of parallel-lying chromonemata. Adjacent discs vary sufficiently in thickness and intensity of staining to form a constant and precise pattern of linear organization that has been utilized by cytogeneticists in the analysis of naturally occurring and induced chromosomal changes. It is generally believed that the extraordinary length of the giant chromosomes is attained primarily by extension of the visible and submicroscopic coils of the chromosomes of the ancestral mitotic cells. According to this view, the Feulgen-positive nodal points along the chromonemata are ultimate chromomeres, and not differentially coiled portions of the thread. However, this interpretation has been contested by Ris and Crouse (333) who suggest that the apparent banding represents only the surface aspect of helically-coiled chromonemata that weave back and forth across the width of the chromosome. These authors state that "the chromonema itself is uniformly Feulgen-positive", but observational evidence lends little support to this interpretation. Since the bands vary greatly in their staining capacity, it follows that the nucleic-acid content of the threads varies from region to region, even if it is assumed that the bands represent adjacent turns of a system of coiled threads. The additional pertinent observation has been made that various protein-staining dyes gives the same differential pattern of banding as orcein and the Feulgen reaction (365). These are indications of regional differentiation, regardless of the interpretation accepted concerning the disposition of the chromonemata. Moreover, since the pattern of banding of a specific region is constant, even when extensive realignment of parts occurs as a result of spontaneous or induced chromosomal rearrangement, it is necessary on the theory proposed by Ris and Crouse to assume that the course of the chromonemata as they traverse the chromosome, and the pattern of association of sister strands at any one level, must be under the precise control of the individual loci in the regions involved. The constancy of the pattern of banding, even when larvae are raised under a wide range of experimental conditions, also indicates that any assumed coiling mechanism which permits such stability is not so sensitive to environmental modification as the mechanism that controls coiling of the chromonemata in plants (*e.g.*, see 409 for a report of the effect of heat on modification of the pattern of coiling in *Tradescantia.*)

The theory that the bands of the salivary-gland chromosome merely represent turns of the chromonematic coils disregards much of the experimental evidence that has been accumulated in recent years. Attention may be called to observations that stretching of the salivary-gland chromosomes of *Chironomus* by micromanipulation involves primarily the interband regions and is accompanied by a decrease in the diameter of the chromosome (36, *cf.* 12, 304, 305). Objections that might be raised to interpretations of chromosome structure based on such fixed preparations have been met by micrurgical studies of living chromosomes of *Chironomus* (82). It was found that chromosomes may regain their initial length after a tenfold elongation, that in the process of stretching the interband regions undergo the greatest elongation, and that the thick bands are more resistant to stretching than the thinner ones but can be separated in some cases into a series of component bands. Fibrils detached from the chromosome in other experiments revealed a pattern of differentiation corresponding to that of the discs of the region from which the strand was removed. Stretching of the chromosome laterally produced a distinctly beaded appearance in bands that were more or less uniformly homogeneous prior to the micromanipulation. These observations and the cytogenetic studies that have revealed the loss or gain of single delicate bands, such as has occurred at the roughest (*rst*) locus in *D. melanogaster* (190), support the theory that the giant chromosome is polytene and that each component strand consists of alternating chromomeric and non-chromomeric segments. The evidence now available is more difficult to reconcile with the theory that these large chromosomes consist of a system of helically arranged chromonemata.

This briefing of the argument must suffice, since a full consideration of any one theory lies beyond the scope of the present article and since another critique has recently been presented (163). In passing it seems desirable, however, to point out that Ris's effort to fit the lampbrush type of chromosome into his general interpretation, by assuming that the side-chains represent loops of a system of coiled threads, likewise disregards much of the experimental evidence. These chromosomes of the amphibian oocyte may be stretched micrurgically without disturbing the specific chromomeric pattern seen in the living condition, or without opening the side loops (110–113). Moreover, the loops, which normally disappear in later prophase stages, may be separated precociously from the main strands that represent the chromatids as a consequence of alteration in pH, changes in salt concentration, the action of proteolytic enzymes, or treatment with X-rays. Evidence of this type led Duryee to the conclusion that the lateral loops grow out from the chromomeres that are distributed along the chromatids. Little support can be derived from such experiments for the contention that "the side branches are the gyres of major coils of the individual chromonemata, which have laterally separated from each other" (332).

Efforts directed toward the denial of chromomeres as definite structural units inevitably emphasize the differences inherent in the more restricted interpretations of the chromomere and the chromonema hypotheses. The assumption that they represent alternative, or mutually exclusive, theories of chromosome organization was a natural consequence of the belief that the longitudinal division of a coiled thread would disrupt the linear order of the genes contained therein. Since that conflict was settled many years ago in a manner that eliminates such objections, it seems desirable to continue to utilize the term "chromomere" to describe the constant, morphologically differentiated segments of the chromonema seen in the living cell as well as in fixed and stained preparations. The further resolution of such regions into tightly coiled threads may be revealed at the microscopic level in some cases; in others the evidence now available suggests that chromomeric organization exists in the fully extended chromonema. As judged by their appearance and staining capacity, the chromomeres can be explained best, at our present level of knowledge, on the basis of the variable capacities of different loci with respect to the timing or amplitude of the nucleic-acid cycle.

Euchromatin and Heterochromatin

Linear differentiation of the extended chromonema is thus revealed in both euchromatic and heterochromatic regions. Accordingly, euchromomeres and heterochromomeres have been described (13), but morphological differentiation is not always possible. In the salivary-gland chromosomes the heterochromatic regions lying adjacent to the centromeres frequently show a vesiculate type of chromomere in contrast with the compact type characteristic of euchromatic regions. As indicated previously, this has not been a useful criterion for revealing the location of intercalary heterochromatic segments in *D. melanogaster;* but, as Schultz noted (271) and as the author has observed in induced rearrangements, the nucleic-acid content of a disc and the appearance of its chromomeres will depend not only on the general environment provided within the nucleus but also on its position within the chromosome. Such position effect might account for the absence of heterochromomeres in regions of the salivary-gland chromosome that, on the basis of their non-specific pairing and frequency of breakage with respect to length, appear to contain heterochromatin. Alterations in more general conditions within the cell may account for the type of flexible heterochromatization reported for *D. palidipennis* (106). In the salivary-gland chromosomes the boundary between heterochromatin and euchromatin is variable; many discs that appear euchromatic in some cells have in other preparations the diffuse appearance characteristic of heterochromatin. Moreover, in mitotic cells the rate of nucleination of the euchromatic portions of the distal section of one arm of the X-chromosome appears to depend on proximity to heterochromatin.

The suggestion has been advanced that the essential difference between euchromatin and heterochromatin may depend on the proximity of chromomeres with the same nucleic-acid cycle, a heterochromatic segment being one with a high proportion of similar or identical chromomeres (308). However, in some plants the heterochromatin in different parts of the same nucleus or in different cells at the same stage may present different cytological appearances (119, 139, review by 320). Cytogenetic studies on *Drosophila* have revealed that certain position effects depend on the proximity of a locus to a specific portion of the heterochromatic material (*e.g.,* 190). In this genus also, marked differences may exist in the salivary-gland chromosomes in the degree of development of regions which appear in mitotic cells to be uniformly heterochromatic. It is known that long segments of chromosomes, or even entire chromosomes, may be represented in the salivary-gland nucleus by very few discs. For example, the heterochromatic segment which constitutes about one-fifth of the left limb of the second chromosome of *D. melanogaster* during mitosis has been reported to be reduced to a single band in the salivary-gland nucleus, whereas the bulk of the heterochromatin seen in the salivary-gland chromosome is derived from another segment of the mitotic chromosome (162). A striking case has been described in *D. nebulosa* (303); one type of heterochromatin shows relatively little "reduction" in the salivary-gland cells in proportion to euchromatin, but another type—not distinguishable from the first by its staining properties in the mitotic chromosome—is represented by a proportionally much smaller number of discs. Following prefixation treatment of root-tips of *Allium* with 0.005 M mercuric nitrate, and other reagents, pronounced differential staining was obtained of heterochromatic regions in the contracted metaphase and anaphase chromosomes. The heterochromatin of the centric regions was found to retain the stain longer in destaining than that in other parts of the chromosome, and the conclusion was accordingly reached that the proximal heterochromatin is of a special kind, essential for the functioning of the centromere (222). (For a fuller consideration of the various types of heterochromatin that have been described, see 320.)

The knowledge of the distribution of heterochromatic regions is slowly accumulating (see 191, 205, 387 for a survey of the various criteria being used in these studies). Further information is also being gained concerning the location and properties of the included genes. The original concept of genetic inertness applied to heterochromatin has been replaced by that of specialized function. Some workers have suggested that heterochromatin may contain a series of replicated units that serve essentially as modifiers of characters determined by other loci (246, 308). Other workers have suggested that heterochromatic genes function primarily in the control of nucleic-acid metabolism.

Chromosomes such as have already been described consist of sections of euchromatin and heterochromatin. Some chromosomes are entirely heterochromatic or nearly so. A classical example, the Y-chromosome of *D. melanogaster*, is essentially "inert" in the sense that it does not carry genes requisite to normal growth and development, but is indispensable because its presence is necessary to insure fertility to the male. The supernumeraries (supernumerary fragments) found in natural populations of several species of plants and animals provide other examples of essentially "inert" chromosomes (87 and 118 present lists of species in which such fragments have been observed). The accessory B-type chromosomes of maize have been reported to show a staining reaction during the resting stage of mitosis that is characteristic of heterochromatin (102), although regional differences in staining capacity are evident at pachytene (231). As many as 34 B-type chromosomes have been observed in the cells of some plants of maize (314) ; in other plants they are wholly lacking. They are often distributed irregularly at mitosis and undergo alterations in size (for a consideration of the possible causes of such changes see 120) ; but nevertheless they are maintained in the population from generation to generation. It has therefore been suggested that their presence confers some advantage to gamete or zygotes that favors their selection (100, 102, 246, 274–276). Under such conditions the supernumeraries can hardly be designated as "inert" in the sense that they are entirely devoid of genic material concerned with nuclear and cellular activity. The opinion has been advanced that the additional heterochromatin they provide permits a more elastic regulation of the nucleic-acid metabolism of the nucleus as a whole than is possible in their absence, or perhaps favors the production of proteins of the histone type (92, 102, see also 50, 54). This explanation is in harmony with the evidence from studies of the effects of extra Y-chromosomes in *Drosophila;* the nucleic-acid content of oocytes in XXY-females is consistently higher than in XX-females (58, 358). In maize, however, it has been found that high numbers of the B-chromosomes cause reduction in vigor and fertility of the plant, and that they can rarely be accumulated in excess of 30 (314). Differential behavior of the supernumeraries may occur in different tissues. In *Sorghum purpureo-sericeum,* the B-chromosomes are lost by lagging in the cells of the radicle before seed ripening and in the shoot tissues as they attain maturity. Chromosomes that reach the cells of the anthers and ovaries, however, are maintained regularly. At the second division in the pollen grain, the B-chromosomes pass to the generative pole undivided. Following this the vegetative nucleus may undergo a series of additional divisions, presumably as a result of the effect of heterochromatin on the cytoplasm (100, 178). In a strain of rye it has been found that the chromatids of the "standard fragments" fail to disjoin at the first pollen mitosis, and pass to the generative pole. A similar process occurs at a corresponding stage in the ovule (277).

Håkansson (157) reports that the supernumeraries of *Godetia nutans,* which he prefers to call "accessories", are not heterochromatic, and cannot for that reason have a significance in the nucleic-acid relationships of the cell. It has been suggested that the accessory chromosomes in this species may have a kind of "parasitic" existence, accumulating as a result of non-disjunction and being maintained because of their relatively harmless effects (291). According to this view, the fragment chromosomes have persisted in the population despite selection that favors plants without fragments.

Supernumerary fragments of varying number and size are also represented in the sex-chromosome complement of the bedbug *Cimex* (89, 386, 388, 389). Probably only two of the three to 15 fragments observed in different individuals have a sex-determining function, the remainder representing supernumeraries. The various possible methods of origin that have been suggested include misdivision of the centromere (89), loss of parts of the X containing the active genes (429), and fragmentation of the X or Y (418, on the basis of a study of the fragments in the reduviid, *Gelastocoris,* which appear to represent nonhomologous sections of an original single X with a diffuse centromere).

In this general discussion heteropyknosis has frequently been mentioned as an index to the location of heterochromatic regions. However, chromosomes that are heteropyknotic in some divisions may condense at a slower rate or at the same rate as the autosomes in other mitoses. The terms "positive" and "negative" heteropyknosis have been applied to these aspects of alteration in the timing cycle. If heteropyknosis is accepted as the sole criterion for identifying heterochromatin, the X-chromosome in certain grasshoppers must be regarded as heterochromatic in spermatocytes, in which it condenses precociously, and euchromatic in oocytes, in which it condenses at the same rate as the autosomes (78). In closely related species of *Gerris,* Geitler (141) observed that the X-chromosome could be either somatically euchromatic or heterochromatic, and concluded on that basis that such heterochromatin could not have an essential physiological function. Among the males of certain scale insects one entire haploid set of chromosomes is heteropyknotic in somatic tissues and during spermatogenesis (165, 350, 351). Probably the entire set is paternal in origin (see 431), having been altered in passing through the gamete so as to produce heteropyknosis in the male but not in the female. Obviously, heteropyknosis in this situation does not provide an index to genic inertness of the chromosomes involved.

SUBMICROSCOPIC ORGANIZATION

An approach to analysis of the finer structure of the chromosome and the distribution of its constituent materials has been made in recent years by various chemical, histochemical and physicochemical methods. A brief summary of some of the contributions will be presented here. Other methods of potential value in the study of chromosome structure have been considered by Loofbourow (228) in his review of borderline problems in biology and physics.

Chemical and Histochemical Analyses

In 1924 Feulgen and Rossenbeck (122) described a technique, now known as the Feulgen or nucleal reaction, for demonstrating the presence of desoxyribose nucleic acid in tissues. Application of this test has shown that desoxyribose nucleic acid represents a major component of the nucleus but not of the cytoplasm; it is detectable in limited quantities in some types of nucleolus, *e.g.*, the salivary-gland cells of *Drosophila* (184). During the growth stages in oogenesis the chromosomes may not give the characteristic reaction, and it has been assumed that they were Feulgen-negative (200), but the work of Brachet (30) suggests that they are never entirely free of desoxyribose nucleic acid, although the degree of dispersion in the large chromosomes may provide only a feeble staining reaction (*cf.* 407). The "in situ" specificity of the Feulgen reaction has recently been questioned (46, 67, 83, 396–399) and defended (11, 32, 33, 40, 56, 107, 370, 401, 402). As the latter group has indicated, the point of view presented by the former workers appears untenable, and more convincing evidence is required if it is to be sustained.

Both desoxyribose and ribose nucleic acids, because of their purine and pyrimidine bases, show maximum ultraviolet absorption at about wave length 2,600 Å. The position in the spectrum of the region of maximum absorption, and its intensity, as indicated by an extraordinarily high extinction coefficient, provide a type of absorption curve that permits detection of nucleic acid in biological systems, even when it is present together with proteins and other substances (47). Thus by comparing the cytochemical evidence obtained by analysis of absorption spectra with that provided by the Feulgen reaction it has been possible to determine the distribution of desoxyribose nucleic acid during the cycle of mitosis. The detection of ribose nucleic acid presents greater difficulties; the criterion of maximum absorption at about 2,600 Å and failure to give the Feulgen reaction is not infallible, inasmuch as purines and pyrimidines may exist in forms other than nucleic acid; *e.g.,* in striated muscle the concentration of adenyl nucleotides is sufficient to permit their localization (61). An additional method of analysis involves use of the enzyme ribonuclease. Structures assumed to contain ribose nucleic acid are tested before and after application of the enzyme, either by measurements of absorption spectra or by staining in Unna's pyronin-methyl green combination (see especially 32). It has previously been pointed out that the conclusions which may be derived from these tests are limited by questions concerning the purity of the enzyme and its specificity of action.

Despite such limitations—and Danielli (83) has pointed out many of the complications inherent in the techniques now used—modern cytochemical methods permit an approach to a quantitative expression of differences in nucleic-acid concentration. They have shown that the quantity of desoxyribose nucleic acid increases during the prophases (48, 49), accumulating locally at each chromomere, with the heterochromatic regions serving as especially potent centers of synthesis (362). During the telophases the quantity of desoxyribose nucleic acid decreases. Evidence has also been obtained that indicates a direct connection between the nucleic-acid metabolism of the nucleus and that of the cytoplasm. A supernumerary Y-chromosome in a female of *Drosophila* increases markedly the ribose-nucleic-acid content of her oocytes as compared with those of the ordinary XX-female (362). Cells of growing tissues exhibit in their cytoplasms an absorption peak around 2,600 Å, characteristic of the cyclic nitrogenous bases present in the nucleic acids, whereas the cells of mature tissues exhibit an absorption spectrum similar to that of the proteins. This has been shown by comparisons of meristematic and differentiated cells in the root tip of *Allium,* and of rapidly growing and inactive yeast cells; by studies of the embryo of rye; and by analysis of embryonic development in the chick (59, 62, 57, 34; see also Brachet's studies on marine eggs, 29). By measuring cytoplasmic basophily following digestion with ribonuclease, it was demonstrated that young pollen-mother and tapetal cells of *Rhoeo discolor* are rich in ribonucleic acid, but that it is used up during the progress of meiosis (296). Accumulation of ribonucleic acid occurs subsequently in the cytoplasm of the pollen grain, presumably as a result of the cytolysis of tapetal tissue. Painter suggests that this accumulation serves to facilitate the rapid synthesis of material during the formation of the pollen tube. In the seeds of *Echinocystis macrocarpa* the giant cells of the nucellus and the endosperm presumably function as the immediate source of the materials necessary for the innumerable mitoses which produce the dense small-celled tissue of the rapidly developing cotyledons of the embryo (366). The phenomenon of endomitosis provides a method for increasing the nucleoprotein content of the nucleus, and the presence of multiple chromosomes or chromosome complexes in a variety of cells with nutritional or secretory functions has elicited various suggestions concerning their rôle. In *D. melanogaster* cleavage mitoses may follow each other within intervals as short as ten minutes, and it has been suggested that the ability of the chromosomes to synthesize the material needed for their rapid duplication is made possible by the reassembling of products, such as nucleotides, that are made available following the disintegration of the nurse cells (299). The question has been raised whether the chromosomes produced endomitotically during oogenesis themselves serve to produce the cytoplasmic precursors (60), since it has been found that in the sea urchin, and in *Drosophila,* the cytoplasmic synthesis takes place before the breakdown of the nuclei. More recently Painter (297) studied the secretory processes in the lateral pharyngeal glands of the worker honey bee, as well as in the salivary glands of the larva of *Drosophila,* and concluded that "endomitotic growth is the cytological mechanism by which heterochromatic centers and nucleolar organizers are increased in gland cells thus making it possible for a single cell to secrete large amounts of protein or other complex substances".

It has been possible to demonstrate in some rapidly synthesizing cells that concentrations of ribose nucleic acid occur around the nuclear membrane and in the nucleolus. In the sea urchin the cytoplasm close to the germinal vesicle shows a much higher nucleic-acid maximum than that in the more peripheral regions (60); and in the nurse cells of *Drosophila* the concentration around the membrane is equally marked (358). Oocytes of the toad likewise show a perinuclear concentration of ribonucleic acid when tested by Unna's stain in combination with ribonuclease (301). In cells of root tips of *Allium* and *Spinacia,* local accumulations of ribose nucleic acid have not been detected in the cytoplasm, but will be determinable only if synthesis exceeds the rate of transport (60). However, the arginine test for protein indicates that during the prophases in root-tip mitoses of *Allium* and *Vicia* there is a synthesis (or differentiation) of basic proteins taking place at the same time that the nucleolus is disappearing (376). The possible rôle of the nucleolus in building up the cytoplasmic nucleic acids has been mentioned previously.

Within the chromosomes themselves quantitative changes in the concentration of desoxyribose nucleic acid have been measured in studies of spermatogenesis in the grasshopper, *Gomphocerus* (48, 49). The amount of nucleic acid was found to be much smaller in the spermatogonia than in cells at early leptotene. Since neither the cytoplasm nor the fluids of the testes showed any appreciable absorption at 2,600 Å during the period of rapid cell division, it was concluded that an active synthesis takes place in the chromosomes. From mid-leptotene to diplotene the quantity of nucleic acid in the nucleus is approximately constant; it appears, therefore, that there is no direct correlation between nucleic-acid synthesis and the chromatid coiling which occurs during these stages. Since the increase in concentration occurs at the time that the splitting of the chromosomes becomes apparent, Caspersson has suggested a fundamental relation between this increase and gene reproduction—in line with a similar suggestion made by Caspersson and Schultz (58), which was based primarily on a study of nucleic-acid metabolism of the salivary-gland chromosome and of the egg cytoplasm in certain variegated races of *D. melanogaster* (see also 92). However, the multiplication of genes and chromonemata apparently occurs one or more mitoses in advance of the time of separation of the chromatids, so that Caspersson's data may merely indicate that the concentration of nucleic acid permits recognition of a previously established line of demarcation between chromonemata (see 171). It has been reported that chromatic materials, when injected intravenously into rats, increase the rate of mitosis in liver following partial hepatectomy (244). On the other hand, the finding that the percentage of histones is low in carcinoma nuclei and chick embryos, and high in resting nuclei, has led to the suggestion that these proteins regulate the growth and division of cells (398).

The relation of nucleic acid to the other components of the chromosome has been studied in a variety of ways. Analysis of absorption spectra indicates that the dark bands of the salivary-gland chromosomes contain high concentrations of nucleic acid, the pale bands much less, but that both contain proteins (47). A trypsin solution to which lanthanum had been added was used to digest the proteins and leave the nucleic acid as an insoluble lanthanum thymonucleate. Other enzyme studies (126–128, 257, 258, 358, 365) have extended these observations. Pepsin did not remove any constituent of the chromosome concerned in maintaining its integrity, although a marked decrease in volume occurred, perhaps owing to removal of the more complex proteins of the matrix. Following treatment with trypsin in the absence of lanthanum ions, the nucleus appeared empty. By the use of nucleases, both the sugar and the nitrogenous components of the nucleic acid presumably were removed; but the continuity of the chromosome was not destroyed, the protein component still giving a characteristic blue color with ninhydrin. It seems, therefore, that the chromosome has a continuous protein structure which is not disrupted when the nucleic acid is split off (258). These experiments indicate that chromosomes contain nucleic acid and proteins in intimate association as nucleoproteins.

An additional series of histochemical tests for localization of cellular components has been developed (31, 32, 366, 372–379). In addition to the ninhydrin reaction, the biuret, xanthoproteic and Millon tests have been utilized, together with special methods for the detection of arginine and tryptophane in sections and entire cells. Nucleic acids have been localized by testing for phosphorus. As an illustration of the method of application: A high concentration of arginine is interpreted as indicating the presence of basic proteins resembling the histones, since they alone—except for the protamines of fish sperms and the edestins found in plant seeds—possess a great amount of this amino acid. Furthermore, histones contain only traces of tryptophane, the more complex proteins a much larger proportion, and this provides a chemical basis for their differentiation. Quantitative determinations proceed on the assumptions that the color intensity is approximately proportional to the amount of the compound present, that the density does not vary greatly in the regions compared, and that secondary color impregnation and adsorption do not occur. The first two involve factors of judgment; the third can be determined experimentally. The arginine test, as used by Serra and Queiroz Lopes, confirms essentially the findings of Caspersson (52), obtained from an analysis of absorption spectra, that the discs in the salivary-gland chromosome contain proteins of the histone type, and probably also proteins of a "higher type", whereas the interbands consist wholly of proteins of the "higher type," perhaps similar to globulin. Tests made on the mitotic chromosomes of the root tips of *Vicia* and *Allium* suggest that the proteins are similar to those found in the discs of the salivary-gland chromosome. The action of nuclease does not diminish the strength of the reaction. The phosphorus test is reported to give a strong reaction in the chromosomes of dividing cells and in spermatozoa, which are rich in nucleoproteins. The detailed procedures utilized in these and other tests for proteins have been summarized by Serra (375); and additional discussion concerning the application of the methods is to be found in other reviews (32, 150, 359).

Considerations such as these lead inevitably to problems of chromosome chemistry and gene constitution (for discussion see 54, 92, 99, 153, 154, 266, 273, 359, 360, 371). The isolation and analysis of parts of the cell by physical and chemical methods (*e.g.*, 71, 108, 256, 267–269, 396) have provided quantitative estimations of amounts of nucleic acids and proteins of various types in chromosomes obtained from normal and malignant tissues. The chemical evidence suggests that the chromosome contains three groups of components—desoxyribose nucleic acids, histones, and tryptophane-containing proteins—the quantities varying greatly according to the type of tissue and its physiological activity (269, 396)*.

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* Since the foregoing was written in the summer of 1946, studies by Mirsky and Ris, and histochemical analyses carried out by the author have confirmed the earlier intimations that the chromosome also contains ribose nucleic acid.

Histochemical tests have also included microincineration and ash analysis. The ash deposits of incinerated chromosomes represent primarily the phosphate residues of the nucleic acid. In the salivary glands the bands differ considerably in their ash content. Details of the methods and their applicability to problems of chromosome structure have been presented in other reviews (116, 420).

Physicochemical Analyses

Birefringence in polarized light, and X-ray and electron diffraction patterns, have been utilized in efforts to determine the submicroscopic molecular architecture of the chromosome.

The use of double refraction of nucleic-acid molecules as a guide to patterns of coiling of the chromonemata (206–208, 210–212, 280, 345, 346) has not provided critical information beyond that available from studies with the ordinary microscope (see 14, 124, 212, 283). Orientation of the thymonucleic-acid molecules, as determined by their birefringence in polarized light, has led to the suggestion that the nucleic-acid chains as well as the polypeptide chains of the giant chromosomes probably lie parallel to the long axis (124, 304, 305, 346, 347; for similar evidence from other methods of analysis see 7, 53, 258, 385). By X-ray diffraction it has been found that the distance between the nucleotides corresponds almost exactly with the backbone spacing (3.34 Å) of the fully extended polypeptide chain of a protein reported by Astbury and Bell (7). The observation that the nucleic-acid chains lie parallel to the long axis of the chromosome contrasts with an earlier interpretation (438) that they are interwoven with the protein chains at right angles to their axis of alignment. A further suggestion that only the protein which is combined with the nucleic acid in the bands is in the fully extended state, that of the interband regions being in the folded state (305), has been questioned (53). No marked orientation of the molecules of thymonucleic acid was detected by means of a sensitive test involving dichroism in the ultraviolet. It thus becomes necessary, in interpreting birefringence of fixed preparations in polarized light, to consider the possibility of artifact resulting from the tendency of the molecules of nucleic acid to become

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The histochemical studies in this laboratory have involved the use of a ribonuclease preparation that had been freed of measurable traces of proteolytic activity through methods developed by Dr. Margaret McDonald.

aligned artificially. (For further discussion of these problems see 348 and 55.)

Since, "in the usual heterogeneous biological material, birefringence is likely to be due simply to orientation of micelles . . .", an attempt was made to employ diffraction methods in the analysis of chromosome structure (37). By painstaking and laborious methods, packets of thousands of salivary glands were obtained from various species of *Chironomus* for X-ray and electron diffraction photography. The results did not warrant formulation of any definite conclusions, but Buck and Melland suggest that the technique has not been tested adequately.

It is by a combination of methods as have been described in the foregoing paragraphs that a more precise understanding will be reached of the sequence of events that leads to alterations in chromosome structure in the cycle of mitosis. Determination of the proportions of the different proteins, their relation to the nucleic acids, and the various degrees of polymerization and depolymerization occurring during the different mitotic phases provides one of the more promising approaches to problems of chromosome structure and gene activity.

CHROMONEMATA

Determination of the number of chromonemata and their patterns of coiling represents the first approximation to an understanding of the method of chromosome duplication. Efforts have been made, therefore, to secure unequivocal fixation images and to discriminate between different observational interpretations by utilizing accessory methods of analysis, such as ionizing radiations. Despite these efforts, conflicting conclusions have often been drawn from different studies on the same organism. Possible differences among species, individuals and types of cells are also complicating factors which prevent formulation of a uniform and general interpretation. Although the studies of recent years have not answered fully all the questions advanced in the 1936 review, several of the observations have provided the information necessary for a critical appraisal of theories of chromosome mechanics based on assumptions of simple numerical relations.

Numerical Relations

Microscopic observations. At the time that the 1936 review was prepared, the evidence derived from cytological observations favored the following interpretation. Intertwined chromonemata seen in early prophase nuclei of somatic mitoses are residual from the chromosomes of the preceding telophase. As the prophases advance, these coils loosen, and each discloses another pair of intertwined chromonemata. Metaphase chromosomes are accordingly quadripartite with respect to chromonemata, and following the separation of the chromatids at anaphase each chromosome reveals two intertwined strands. Since premeiotic anaphases and telophases showed the same type of organization, it was assumed that the leptotene threads of meiosis were longitudinally double, although appearing unsplit under the microscope. Pairing of these threads and subsequent appearance of a tertiary split accounted for the octopartite chromosome observed at metaphase of the first meiotic division. Anaphase chromosomes of this division would accordingly have four chromonemata, those of the second meiotic division two.

More recent observations, although differing widely with respect to such details as the time of splitting of the chromosome, have confirmed the existence of multiple chromonemata at the various stages of somatic and meiotic mitoses (*e.g.*, 1, 2, 8, 23, 42, 75, 80, 104, 131, 132, 134, 140, 169, 202, 207–209, 212, 221, 236–239, 241–243, 278, 279, 281, 285, 318, 319, 334, 335, 391, 424, 425, 436, 437, 439).

Among the clearest illustrations of coiled chromonemata are those obtained by treatment of smears of microsporocytes with weak solutions of acids, alkalies and other agents preceding their fixation (*e.g.*, 79, 95, 214, 251, 293, 380). Application of such treatment to the growing pollen tube, whose mitotic chromosomes are readily reached by the reagents, has provided fixation images that indicate the presence in each metaphase chromatid or anaphase chromosome of a tightly coiled thread rather than loosely intertwined chromonemata. Some workers have maintained that the coil is composed of an undivided strand, an interpretation that stems from the concept that the chromatid, which behaves as a unit in mitosis and in crossing over, is likewise the fundamental structural unit (see 87 for exposition of this viewpoint). The thread, even when extended, may appear single, but this can hardly be accepted as conclusive evidence that it is undivided or that well defined cleavage planes for succeeding mitoses are not determinable. In somatic chromosomes, split ends or bipartite satellites, which are occasionally observed during anaphase, furnish evidence that the thread is divided (*e.g.*, 75, 137, 185). Since such observations were made on chromosomes that had not received prefixation treatment as well as on those that had, there seems no valid basis for the interpretation (432) that the appearance of duality following certain types of prefixation treatment is attributable to the action of the agent and does not reveal the normal pattern of organization of the chromosome. In the living germinal vesicle of the frog each of the homologues appears as a single thread under the conditions of microdissection, although the tetrad nature of the bivalent is implied by the presence of chiasmata, and four chromonemata may be revealed if the cells are fixed, or treated with X-rays (113). It has been suggested that dehydration of the nucleus may cause the close approximation and optical obliteration of the individual strands of a complex spiral, and that "reversible gelation-peptisation changes", especially in the matrix of the chromosome, would account for the variable expression of a prophasic or telophasic split (209, 214).

The compounding of several strands into an optically single one is strikingly represented in the chromosomes that presumably originate by repeated duplication of the chromonemata without intervening mitosis. In the epithelium of the hind gut of the larva of the mosquito, *Culex pipiens,* each chcromosome may be composed of from two to 32 strands united in a common centric region and frequently appearing under the microscope as a single thread rather than as an aggregate (19–22, 151, 152). Similarly, the origin of the giant chromosomes of the salivary glands of the Diptera has been traced to the fusion of a pair of homologues, each of which is longitudinally double (35, 298). Nevertheless, in the smaller cells of the salivary gland the chromosomes appear single (263) ; in the larger cells the homologues are easily distinguishable in unsynapsed regions, but only rarely are indications of the fundamental quadripartite structure detectable (125, 195, 259, 298, 417).

When cells constitute masses of tissue to which the reagents do not have immediate access, the problem of securing adequate fixation becomes more difficult. Following prefixation treatment with a dilute solution of sodium cyanide (75), a tightly coiled spiral was observed in chromosomes of the premeiotic and root-tip metaphases of the cultivated tulip, and also in metaphase and anaphase chromosomes of the second meiotic division in *Gasteria.* Because of such observations (see also 95, 137, 139, 140, 342, 421) some workers regard the interlocking double-coil type of structure so frequently described by plant cytologists as a fixation artifact. Others, proceeding on the assumption that the appearance of the single-coil type of structure does not imply that the chromonema is undivided, have suggested that the transition from one type to the other might conceivably follow a change in the form of the chromosome, a redistribution of its materials or an alteration in the expression of the standard and subsidiary coils (see 209, 213, 260, 283, 319). Observational evidence verifying the existence of the interlocking-double-coil type of structure continues to accumulate. By treatment of root tips of *Allium* with salts of heavy metals prior to fixation it was possible to detect intertwined chromonemata in each metaphase chromatid (221). "The half-chromatids often form a relational spiral of great clearness", and the space between them is sometimes so considerable that "the phenomenon cannot be due to an optical error". Moreover, metaphase and anaphase chromosomes of microspores of *Trillium* show closely coiled somatic-type spirals, each of which is composed of two chromonemata that in certain regions "can be seen to be wound in the form of a plectonemic spiral" (391).

Some evidence has been presented in favor of the view that the somatic telophase chromosome contains at least four microscopically detectable chromonemata (2, 209, 214, 239, 282, 283, 285–287). Determination of the number of strands by cytological methods involves interpretation of diffraction patterns that are made more confusing by uncertainties concerning the helical relationships of sister chromonemata and the possible existence of a minor gyre along each of the more conspicuous coils. Use of the ultraviolet microscope may possibly afford some advantages in resolving these details. Photographs made with this instrument suggest that late-anaphase chromosomes in germinating spores of the fern, *Todea,* have four differentiable chromonemata (239).

Four coiled chromatids can readily be demonstrated in first meiotic metaphase chromosomes of microsporocytes of various species of plants. Further subdivision into half-chromatids, which will separate at the first pollen grain division, has been detected in some species with large chromosomes (for summaries of earlier work see 185, 209, 283). Physically complete separation of meiotic half-chromatids occurred in plants of *Trillium kamtschaticum* that

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Studies utilizing radiations. Treatment of chromosomes with X-rays has been utilized in an effort to discriminate between the divergent interpretations of the descriptive cytologists. The first experiments proceeded on the assumption that irradiation of the divided, or double, chromosome would produce breaks independently in the two chromatids (chromatid breaks), whereas irradiation of the undivided, or single, chromosome would produce breaks that would be transmitted with the subsequent division equally to the daughter chromatids (chromosome breaks). It was assumed that the energy required to induce breakage is derived from a single ion pair, and that, following the longitudinal division of the chromosome, the sister strands would soon become separated so widely that they could not be included within the sphere of action of a single ionization—a sphere whose radius could hardly extend over microscopically detectable distances (103). In fact, the experimental results indicated in a general way that prior to a certain stage in the course of mitosis—either in interphase (245, see also 63) or early prophase (330, see also 43, 44, 337)—the chromosome reacts as a unit in response to the ionizing radiation, and that subsequently each of the two chromatids is affected independently. It remained a question, however, as Huskins and Hunter pointed out in 1935, whether this reaction represented in all cases a valid guide to structural singleness and doubleness, and whether two or more chromonemata might not act as a unit in response to the radiation. In addition to the cytological observations that lent support to this possibility, types of induced aberrations had been observed whose production appeared to depend on the simultaneous breakage of two adjacent chromatids (281).

Additional evidence was subsequently obtained from studies on *Tradescantia,* in which the processes of breakage and recombination occur in comparatively rapid succession (216, 339) and in which the splitting of the chromosome is presumably effected in a comparatively short time (341). Irradiation of the prophase nucleus of the first pollen-grain mitosis has yielded shortened, dicentric chromosomes and accompanying U-shaped acentric fragments whose production is attributed to the fusion of ends of sister chromatids broken by a single "hit" (341). Additional evidence that microscopically differentiable sister chromatids may be severed conjointly is provided by X-ray-induced double deletions having corresponding parts detached from both chromatids (338, 411). Aberrations of this type are comparatively infrequent in cells rayed at late prophase, but more abundant following irradiation at early prophase when the chromatids are closer together. Moreover, the frequency with which they are produced is proportional to the dosage, as might be expected if the breaks involved were produced by a single ionizing particle. If these same stages are treated with very soft X-rays (64), or ultraviolet rays (408, 411; also see 395), single chromatid deletions rather than isochromatid breaks are produced almost exclusively. Such results suggest that there is a marked qualitative difference between the effects of different kinds of radiation, the sphere of action of the very soft X-rays and the ultraviolet quanta rarely including more than one strand of a divided chromosome, that of the harder X-rays often encompassing both.

The path to an understanding of such results was indicated by studies of neutron-induced rearrangements (146, 147, 416). It was found that neutrons are effective in inducing two-break chromosomal rearrangements with a frequency proportional to the dosage. This indicated that the densely ionizing track of a single recoil proton causes breakage of two separate chromosomes, which it traverses successively. By analogy, Lea and Catcheside (216) reached the conclusion—which is fairly well substantiated (*e.g.,* 117)—that X-rays may produce isochromatid breaks by the action of a single secondary electron, which crosses successively the two chromatids even when they are widely separated. Thus the progress of splitting of the chromosome appears to reduce the chance of simultaneous breakage of the two strands, not by removing them from a single possible sphere of action, but by reducing the chance that both will be hit by a single electron in rapid succession. It was also necessary for Lea and Catcheside to modify earlier conceptions of the breakage process by postulating that it is not dependent on a single ionization, even when a single strand is involved, but on a dense cluster of perhaps 20 ionizations within the thickness of the strand. Such clustering takes place not only at the tail end of the electron track, or at places where the main track branches out into other short tracks, but along the entire length of the track of a recoil proton.

Studies of the effects of different types of radiation on chromosomes in comparable stages of division have lent support to some of the earlier observations that suggested the occurrence of aberrations involving half-chromatids (*e.g.,* 43, 44, 243, 282, 283, 287). In his study of the differential sensitivity of prophase pollen-tube chromosomes of *Tradescantia* to X-rays and ultraviolet radiation, Swanson (411) found ultraviolet-induced breaks extending only part way across the diameter of the chromatid, suggesting that only one of two half-chromatids had been broken. He also observed an unequivocal case of a half-chromatid translocation induced by X-rays: "the half-chromatids at the point of breakage could be traced with ease and clarity".

The evidence provided by the studies of *Tradescantia* leads to the question whether the chromosome and chromatid breaks obtained following irradiation of the spermatozoa of *Drosophila* may likewise be derived from chromosomes that are longitudinally divided. Since the individual chromosomes are not identifiable in the spermatozoon, and since the induced breaks do not recombine until after the sperm has entered the egg in the process of fertilization (189, 272), the actual time of division of the chromosome into chromatids remains unknown, and the probability must also be considered that the presumptive cases of chromatid breakage actually result from differential recombination of sister chromatids that were effectively separated after the time of irradiation. The significance of the various chromosomal mosaics and duplications in evaluating these alternative interpretations has been discussed at length (*e.g.,* 188, 272; see also 65 for a consideration of the ring-X chromosome). Fractionals or mosaics for changes that presumably represent point mutations may also be produced by irradiation of the spermatozoa of *Drosophila,* and it is especially difficult to reconcile the production of some of the observed types with the theory that subdivision of the chromonema has not occurred prior to the time of treatment.

From the various considerations presented in the foregoing paragraphs it is apparent that the methods of radiation biology tend to support rather than deny the interpretations of chromosome structure derived from the major portion of the cytological observations. Analytical procedures have in general involved treatment of cells assumed to be in a certain stage of mitosis and observation of the induced changes in the cells at a considerably later stage. It is to be hoped that some of the ambiguities and uncertainties inherent in this indirect method of approach may be avoided by direct observations of the effects of ionizing radiation on chromosomes, utilizing such techniques as Carlson (44) has developed for the study of living neuroblasts of grasshoppers. There is the further possibility that a comparative study of X-ray-induced and chemically induced gene and chromosomal mosaics—such as Auerbach (9) proposes—may cast additional light on the structure of the chromosomes at the time of treatment.

Mechanism and Patterns of Coiling

Patterns of coiling. Interpretations of methods of coiling have varied according to the premises accepted with respect to the number, disposition and organization of the spiral threads at the various stages of mitosis. Earlier efforts to envisage a simple mechanism whereby a relatively straight thread might assume a helical form to facilitate distribution of the chromosomes on the spindle, soon met with such difficulties as changes in direction of coiling within a chromosome arm, persistence of some degree of coiling throughout the cycle of mitosis, and occurrence of closed or ring chromosomes in which multiplication of chromonemata and separation of chromatids could occur without their entanglement. As a result, a series of critical studies of patterns of coiling have been carried out in recent years, using the favorable material provided by large plant chromosomes. Many of the observations have been evaluated in other publications (*e.g.*, 170, 394) and need not be presented in detail at this time.

During the early prophase of somatic mitosis in plants with large chromosomes, the chromonemata are seen in loose spirals with wide gyres. These are the relic coils derived from the standard coils of the preceding mitosis. As the prophase advances it can be observed that the chromatids, which are contorted into these wide loops, are closely twisted around each other, or relationally coiled. Their constituent chromonemata are helically disposed, forming the so-called standard coils (283). The existence of a subsidiary coil along the standard coil has been inferred from observational and experimental evidence (*e.g.*, 95, 98, 260).

The chromonematic coils are especially conspicuous during meiosis, and have been studied extensively in the chromosomes of *Tradescantia* and *Trillium*. Each of the chromatids of the bivalent of *Tradescantia* at first meiotic metaphase is coiled into five to eight major gyres. The degree of coiling is under genetic control and therefore varies in different species and their hybrids; it is modifiable by environmental variables such as temperature (see 98, 409 for summary of earlier studies). Along the major coil there is a small-gyred minor coil (79, 170, 209, 293). Swanson (409) even reports the presence of a subminor coil, but since he regards chromomere-like aspects as evidence of minute coils, his observations must await confirmation. In *Trillium* the minor coils are apparently less conspicuous. Photographs suggesting their existence have been published (79, 176, 384), although in some preparations only a waviness rather than a tight coiling is apparent along the wide-gyred spiral (170). The observed divergency in form is probably not of fundamental importance but attributable to variable conditions existing during the spiralization cycle (208).

The two chromatids of each dyad are intimately paired in *Tradescantia,* whereas in *Trillium* each is a relatively independent coil. These chromatid major coils in *Trillium* are clearly paranemic (this type of coil, also called an "anorthospiral", permits separation without interlocking). When first-meiotic-metaphase chromosomes of *Tradescantia* were artificially uncoiled by ammonia (reported in 208), the chromatid major spirals were interlocked, as if derived from a plectonemic, or orthospiral. However, if the two chromatid major coils were normally paranemic, and the treatment causes uncoiling by rotation, that would account for the intertwining (170). It is certainly clear in smears of microsporocytes of *Tradescantia* (unpublished data of the author) that the chromatids constituting the dyad at first meiotic anaphase are free to separate—and can be forced apart when the pressure used in smearing is sufficient to crush the cell—without any apparent loosening or uncoiling of the major gyres. This is evidence of a paranemic arrangement; and there is no convincing observational evidence that a plectonemic arrangement existed during earlier stages and became transformed to the paranemic type by breakage and reunion of chromatids (crossing over, as postulated in 252).

Indications of separation into half-chromatids are sometimes apparent in condensed chromosomes at metaphase, but are seen most clearly following artificial uncoiling of the major spiral (208) or in temperature-treated material (170, 173, 394, 409, 412). When clearly defined, the half-chromatids of *Trillium erectum* appear to be arranged in a plectonemic coil (170). On the contrary, in heat-treated material of *Trillium* and *Tradescantia* lateral separation of sister half-chromatids occurs without entanglement (255, 409). The free separation indicates a paranemic relationship of the sister strands, although it may possibly have been derived secondarily as a result of rotation induced by the treatment. It is particularly important to note that in *Tradescantia* (410), as in *Trillium,* the microspore chromosomes show plectonemic relational coils. In the grasshopper, *Romalea,* at first meiotic anaphase each of the chromatids of the X-chromosome reveals two half-chromatids which are loosely coiled around each other and presumably have been derived from a plectonemic coil (265).

During interkinesis in *Tradescantia* the major coils of the chromatids relax considerably, the spirals of the second division resembling those of somatic mitosis. In *Trillium* there is practically no interkinesis, and the chromosomes pass relatively unchanged from the anaphase of the first division to the metaphase of the second. The major coil of second anaphase is almost as large-gyred as that of the first (172, 249, 435). That its chromatids are plectonemically arranged was determined by direct observation and by analysis of relic and relational coiling of the first microspore division (391, 394).

The sequence of changes by which the conspicuous meiotic coils of metaphase and anaphase are derived from the attenuated leptotene chromosomes can not readily be detected cytologically. Inability to follow the coils from the time of their inception constitutes a severe handicap in the analysis. As indicated previously, interpretations have not agreed that the chromomere-like bodies of leptotene represent minute coils along the chromonemata. It has generally been assumed that new coils are not formed until full extension has eliminated the spirals of the premeiotic anaphase chromosomes and the homologues have been associated throughout a part or all of their length (*e.g.,* 175, 236). Studies of normal and heat-treated material of *Tradescantia* suggest that the minute gyres that appear in early zygotene, or perhaps even in leptotene, are destined to become the major coils of metaphase by a process of despiralization which involves reduction in chromosome length and gyre number and increase in gyre diameter. During the later prophases the minor coil makes its first visible appearance, becoming increasingly evident as anaphase approaches (409, 410, 412; *cf.* 206–209 on *Tradescantia,* 212 on *Trillium*). Other observations have been interpreted as indicating that the minor coils precede the major ones in their formation (*e.g.,* 336 on *Tradescantia*). In support of this interpretation it has been reported that during mid and late diplotene in *Trillium* there is present only a small-gyred spiral with some intimations at the ends of the chromatids of the beginning of the larger-gyred coil (79).

Direction of coiling. An extensive series of observations on the direction of coiling serves as a further basis for evaluating the various hypotheses that have been advanced to explain the mechanisms involved. In somatic chromosomes it is only rarely possible to map accurately the course of the chromonemata through successive gyres. When the gyres are few in number, as in the flagellate, *Spirotrichonympha,* or in spermatogonial chromosomes of grasshoppers, occasional reversals in direction along the chromosome have been observed (72, 428). The chromonematic spirals in somatic chromosomes of plants are generally more compact and greater in number (20–25 in *Tradescantia, ca.* 80 in *Fritillaria*). Occasional preparations of metaphase and anaphase chromosomes reveal clearly the direction of coiling (336), but studies have more generally been made of the system of relational coiling during the late prophase stages. These observations suggest that the direction is essentially at random for the two arms of a chromosome and for corresponding arms of homologous chromosomes. There is a tendency for the coiling to be in the same direction from the centromere to the distal end of the chromosome, although reversals do occur (336; *cf.* 85). Sparrow (391) suggests that reversals observed in the somatic-type coils of the microspore chromosomes of *Trillium* may play a part in maintaining uniformity of coiling along the chromonema and in facilitating the untwisting of prophase relational coiling.

Explanations of the origin of the relational coiling observed in somatic prophases vary according to the premises accepted with respect to the number and relation of the chromonemata during anaphase and telophase (see 209 and 213 for an extended discussion of the alternative possibilities; also see 391 for a listing of the proponents of the various interpretations). Darlington (85, 87) contends that the "relational coiling" of the chromatids seen in early prophase results from the division of the chromosomes at the end of the resting stage along a cleavage plane that will enable the chromatids to lie parallel at metaphase and separate freely at anaphase. This coiling, which compensates for the relic coiling and is therefore necessarily in the opposite direction to it, presumably disappears along with the relic coiling during prophase. The relational coiling seen at late prophase and metaphase is attributed to the chromatids' being dragged round one another as a consequence of forces determined by the assumption of an internal spiral in each chromatid. Consistency in direction of coiling within a chromosome arm, arising from an internal molecular torsion, is a basic tenet of this hypothesis (however, see preceding paragraph for evidence that the direction is not always consistent).

Twisting of chromatids, presumably in connection with the origin of new spirals, must also be considered as a mechanism involved in the production of prophase relational coiling, if it is assumed that two chromatids arranged in the form of a paranemic coil are present in the anaphase chromosome; for, under such conditions, extension of these coils will provide strands that are not interlocked, and intertwining must occur subsequently. The sequence of events would thus parallel in a general way that reported for diploid somatic mitoses of Diptera, in which somatically paired but independent telophase homologous chromosomes become intertwined during the resting stage, presumably in relation to chromosome reduplication (151, 152, 390). If, however, we accept the evidence, previously outlined, that the anaphase chromatids form either a loose or a compact plectonemic coil, then the intertwining observed during the prophases may be regarded as residual from the preceding mitosis (*e.g.*, 182; for discussion see 209, 394).

A further question arises concerning the pattern of association of the half-chromatids in those anaphase chromosomes reported to have four closely associated chromonemata. Some observers suggest that the arrangement is paranemic (239, 285). As visualized by Kuwada (209, 213), the two chromatids of the anaphase chromosome run parallel or twist around each other to some degree, each being composed of plectonemically arranged half-chromatids. In the untwisting of half-chromatids in the prophase, the two chromatids become interwined, since it is assumed that the half-chromatid spirals are to a large extent coiled in the same direction. Studies of the multiple-complex cells of *Culex pipiens* indicate that several chromonemata may exist in a complex plectonemic relationship and subsequently dissociate freely. The relational coiling between sister chromonemata is presumably established at the time of their formation, and at the same time there occurs a loosening of the relational coiling between the strands that originated at an earlier division. Some descriptions of the behavior of chromonemata in an ordinary somatic mitosis conform to such a general pattern; the anaphase chromatids form a plectonemic spiral; and their relational coiling is reduced gradually during the prophases when the plectonemic coils of the half-chromatids first become clearly defined. Whether the parallel thus drawn represents more than a superficial analogy depends on the validity of the underlying assumptions concerning the number of chromonemata and the origin of relational coiling in association with chromonema duplication.

The relational coiling derived from the somatic coils of the last premeiotic anaphase is presumably eliminated during the early meiotic prophases by the straightening out of the chromosomes (410). Kuwada (209) has suggested that synapsis takes place between homologues while they are unwinding the relational coils derived from the paired half-chromatids of the last premeiotic anaphase, and that when such untwisting occurs in parts of the homologues that happen to be twisted in the same direction, intertwining of the homologues will result, with possible chiasma formation at the points where the threads cross each other. Other evidence indicates that the threads are more or less fully extended and lie essentially parallel at the time of synapsis (*e.g.*, 254). The relational coiling between homologous chromosomes at pachytene has been attributed by Darlington (84, 87) to an internal molecular torsion, and the suggestion advanced that chiasmata may replace relational coiling at the time that the chromatids are assumed to divide at the end of pachytene. However, as indicated previously, the basic assumptions on which this theory rests are open to serious criticism. If relational coiling at meiotic prophase is responsible for crossing over, and is the same in origin and nature as the relational coiling of mitotic prophase, a correlation might be expected to exist between the amount of relational coiling per unit of chromosome length and the number of chiasmata. Analyses carried out on the chromosomes of *Tradescantia, Vicia* and *Allium* (175, 336), and on a series of species of grasshoppers (81), suggest that such a correlation may possibly exist, although the data do not permit any definite conclusions.

The direction of coiling of the major spirals of the first meiotic division has been determined in a number of species. Changes in direction occur more commonly at the centromere, but also at chiasmata and other intercalary regions. The direction is essentially at random for homologous chromosomes. In *Vicia faba* and in *Tradescantia,* reversals were observed at chiasmata and occasionally at other loci (336). An extensive series of observations on synaptic, desynaptic and asynaptic chromosomes of *Trillium erectum* (170, 173, 394) led to formulation of the following hypotheses: *a*) The direction of coiling is random on either side of the centromere; this will cause half as many changes in direction as there are chromatid attachments. (Occasional cases of non-random change across the centromere were attributed subsequently—392—to a disproportionate or non-random untwisting in chromosomes with interbrachial reversals, and not to coiling that was non-random at its inception.) *b*) Chiasmata may cause changes in direction at a rate equal to the chiasma frequency—the basic assumptions here being that coiling begins after chiasma formation and that direction of coiling is random with changes optional rather than obligatory at the chiasmata (however, *cf.* 247, 248). *c*) Other reversals of direction ("adventitious reversals") occur with a frequency that is proportional to chromonema length and gyre number.

The frequency of changes in direction in a limited number of second-anaphase and microspore-relic coils in *Trillium* was found to correspond closely with that of first anaphase (394). Other analyses of relational coiling in gametophytic divisions have been made on *Tradescantia paludosa, Allium cepa, Vicia faba* and *Hyacinthus orientalis* (174, 175, 423).

Mechanism of coiling. In the preceding paragraphs it has been shown that reversals of direction of coiling within a chromosome limb may occur in root-tip, meiotic and postmeiotic mitoses. Earlier studies (summarized in 185) have indicated that the direction of coiling of the minor spiral may be the same as or different from that of the major one. These observations run counter to any simple explanation of the mechanism of coiling which assumes a dependence on internal torsion directed from the centromere (*e.g.,* the hypotheses of Darlington, 84, and MacKnight, 234). Svärdson (405), on the basis of observations of changes in the relative lengths of the two arms of the chromosomes of salmon in the course of a mitosis and in consecutive mitoses—the effect being more marked in the longer arms than in the shorter arm—suggested a "migration" of spiralization over the chromosome with successive impulses originating from the centromere or from the telomere (terminal centromere). Such a mechanism would permit occasional changes in direction but does not account for a distribution that is proportional to chromonema length. A further suggestion that the distal portion of the chromosome may initiate the coiling process has been made by Coleman and Hillary (79) on the basis of their observation that the major coil of *Trillium erectum* is first detectable in this part of the chromosome. "Forces developing in the chromonema itself . . . independent of any forces exerted by the matrix" are assumed to cause spiralization. Nebel (284) has supplied a hypothesis which retains the molecular spiral envisaged by Darlington but endows it with properties that permit the direction of coiling to be facultatively determined anew at each period of gene multiplication. It is suggested that the gene strand is made up of "nemameres", intermediate in size between the gene and the chromomere, regionally differentiated in such a fashion that torsional repulsion permits a randomness of direction of the molecular spiral. Random changes in direction of coiling within a chromosome arm can be explained on the basis of this hypothesis, but the concept that reversals can take place only concomitantly with nemameric reproduction is not in accordance with the fact that there is a correlation between chiasmata and changes of direction.

Other theories have been formulated on the general assumption that an elastic chromonema is thrown into a helical form as a result of being confined within some encasing material. Kuwada (207) proposed the theory that anisotropic swelling causes an intensification of the internally directed tendency of the chromonema to twist, and that when imbibition water is distributed evenly the threads are forced to untwist. The untwisting involves transformation of the twist into a spiral which assumes a regular and compact form as a consequence of the contracting force of the matrix. According to this theory the direction of the internal twist is the same as that of the spirals themselves, the twist and spiral being "different morphological expressions of the same twisted condition" (*cf.* Darlington's molecular-spiral theory which assumes an internal twist that is compensatory for and in the opposite direction to that of the larger spirals). Matsuura (254) argues that the properties of the matrix alone can not account for a uniformly regular spiral as is seen in *Trillium.* He concedes that the matrix imposes space limitations but believes that the elasticity of the thread, and its electrical charges, influence the pattern of coiling. Sax and Humphrey (340) had suggested earlier that contraction or compression of the two flexible chromatids without rotation within the chromosome pellicle would account for the primary coils of meiosis. This interpretation has been contested (435) on the grounds that the required shortening in the over-all chromosome length does not occur between late diplotene—when the major coil is first laid down—and anaphase (see also 79). However, this criticism is based on a study of *Trillium* which may differ markedly from *Tradescantia* in this respect. Observations in general suggest that a reduction of the over-all chromosome length occurs between pachytene and metaphase—to about one-ninth in *Tradescantia* (342) and to about one-fourth in *Osmunda* (240). The theory of Sax and Humphrey has also been contested on the grounds that the chromatids do not necessarily coil together but may coil independently (410). Wilson and Huskins have in turn advanced the theory that coiling results from elongation of the chromonema within a confined space bounded by the pellicle. Measurements on *Trillium erectum* indicate that elongation of the chromonema occurs between early diakinesis and first anaphase, and between second anaphase and microspore prophase (391, 394). Contraction occurs between zygotene and early diakinesis, between first and second anaphase, and during microspore prophase. It is suggested that the chromonemata increase in length within a matrix that is neither expanding nor contracting longitudinally, although capable of increasing in diameter, and that they are forced into a spiral thereby. Reversals of direction may be due either to the effect of "bends in the pellicle" or to the fact that coiling begins at various places along the chromosome. Objections to this theory have been made on the grounds that the inception of the major spiral is at the ends of the chromatid, not at random along its length (79), and that assumed changes in length may be referable to alterations in the amplitude of the minor spirals rather than to pronounced modification of the real length of the chromonema. Measurements made by some investigators have indeed suggested that the length of the chromonema remains relatively constant during the various phases of mitosis (236, 279, 302). Decisions here depend on the ability to follow precisely the turns of spirals, which lie close to the threshold of microscopic visibility.

Clarification of these details should lead to a more rigid definition of the coiling process in terms of molecular patterns, although conceivably the physico-chemical approach may provide the clue for integration of the microscopic observations. Some efforts in this direction have already been recorded. Elvers (116) has advanced a hypothesis based on the existence of nucleic-acid-charged chromomeres in the form of dipoles oriented across the chromosome, the attraction between poles of adjacent chromomeres causing spiralization. Sparrow (393) has observed that reduced spiralization follows X-ray treatment of meiotic-prophase chromosomes, and has suggested that the effect may be due to an induced deficiency of desoxyribose nucleic acid. Its normal increase in prophase would, in this case, play a rôle in the mechanism of spiralization. It has been demonstrated by Astbury (6) that changes in length of protein fibers may follow folding and unfolding of the polypeptid chains. Östergren (290) has recently suggested that folding of this type, rather than increased spiralization, may be responsible for the excessive contraction of chromosomes induced by cold, colchicine and other agents. In this way fibrous protein chains are brought to a more or less corpuscular form, the contraction being manifested directly in the contraction of the chromosome. The reverse process of unfolding of submicroscopic convolutions has been visualized as a possible cause of chromosome elongation in leptotene (236) and as a basis for the great increase in length attained by the giant chromosomes of the Diptera. However, Wilson and Huskins have pointed out that reversible changes in length can take place in fibrous proteins, such as keratin and myosin, without any evidence of intramolecular reorientation appearing in the X-ray diagrams.

From all these considerations it is apparent that the existence of a universal coiling mechanism has not been established. The evidence in favor of a torsional mechanism seems most convincing, especially in the light of the observations that once the chromonematic coils have been established, in the mitotic chromosomes or in the endomitotically produced complexes, there is a progressive reduction in their number by a process of continuous despiralization. Reversals in the direction of coiling are difficult to harmonize with a torsion mechanism unless control in localized regions permits orientation of blocks of smaller or greater size. The basis for such differentiation is in turn not apparent. There also remains the problem of the ring chromosome whose chromatids are frequently able to separate at anaphase and therefore must have been lying parallel without any interlocking. If it is assumed that a plectonemic coil exists from the time of the inception of the ring, there remains the problem of reducing the consequent relational coiling so as to permit free separation of chromatids. Rotation of chromatids at the centromere, or breakage with subsequent attachment, would provide possible methods of relieving the strain set up in such a relationally coiled system at the time that new coils were being enlarged. Of 65 X-ray-induced rings observed at metaphase and anaphase in microspores of *Tradescantia,* only 10 were "disjunctional", permitting free separation of the chromatids; the remainder had interlocked chromatids or they had opened out to form a dicentric ring (174). Since the X-ray treatment was given during the resting stages or early prophases, these data suggest that the chromatids do not rotate at the centromere or break during the course of the prophase stages. The dicentric rings are similar to those of *Zea* studied extensively by McClintock (summary in 233), which presumably arise as a result of a single exchange or crossover between the two sister chromatids. The continuous, double-sized, dicentric ring breaks in anaphase as a result of the strain imposed by the movement of the centromeres toward opposite poles of the spindle. There are ring chromosomes, however, such as those of *Drosophila melanogaster,* which, although capable of forming dicentric rings, are nevertheless perpetuated in unaltered form through successive generations (65, 364). Little is known of their pattern of coiling aside from the casual statement of Schultz and Catcheside that they have seen no difference between the spirals in these closed X-chromosomes and those of the rod X. It has been suggested that the ability of ring chromosomes to separate indicates that relational coiling may be facultative rather than obligatory (85). A study of patterns of coiling in chiasma loops of *Trillium* led to the conclusion that when strands are "fixed" at their ends they are unable, if below a certain length, to assume regular cylindrical spirals, but merely assume corrugated configurations (254). Whatever the "compensatory" mechanism for relational coiling may be, it does not lead to interference with the orderly progress of mitosis or crossing over in ring chromosomes. Certainly a more precise description of the cycle of coiling in these forms is desirable. Perhaps the newer methods of microscopic analysis outlined in the introductory section of this review will provide a further step in securing the basic data required.

SUMMARY

In this review an effort is made to indicate the present level of our knowledge of chromosome structure, and prospective approaches to further studies. Morphological considerations based on observational evidence have been supplemented in recent years by various chemical, histochemical and physico-chemical methods of analysis, using large plant chromosomes and the giant polytene chromosomes of the salivary glands of the Diptera. The findings are appraised in so far as they bear on such particulars as linear organization, mechanism of coiling and relation of the chromonemata to other materials of the chromosome.

The compact cylindrical chromosome contains within its limiting membrane the helically coiled chromonemata and the associated matrix. Consideration of the evidence available from physical and chemical methods of analysis, together with the older morphological considerations, leads to the suggestion that the term "matrix" should be reserved for the Feulgen-negative portion of the chromosome which surrounds the chromonemata and which can be dis-

sociated from them by various techniques. The linear differentiation of the chromosome is discussed with respect to constrictions, chromomeres, and euchromatic and heterochromatic regions. The term "chromomere" is used to describe the constant, morphologically differentiated segments of the chromonema seen in the living cell as well as in fixed and stained preparations. The further differentiation of such regions into tightly coiled threads has been revealed microscopically in some cases; in others, the evidence now available suggests that the chromomeric organization exists in the fully extended chromonema. Evidence concerning the distribution of heterochromatin among the members of the chromosome complex is considered, and the possible rôle of this material in the mitotic cycle is outlined.

Analysis of the finer structure of the chromosome and the distribltion of its materials has involved the use of monochromatic ultraviolet radiation and various chemical tests for identification and determination of the distribution of cellular components. These cytochemical methods have permitted an approach to an expression of differences in concentration of nucleic acids and proteins, and have suggested possible relations between the nucleic-acid metabolism of the nucleus and that of the cytoplasm.

Determination of the number of chromonemata and their patterns of coiling has involved efforts to secure unequivocal fixation images and to discriminate between different observational interpretations by accessory methods of analysis, such as ionizing radiations. The observations, although differing widely with respect to such details as the time of splitting of the chromosome, have confirmed the existence of multiple chromonemata at the various stages of somatic and meiotic mitoses. On the basis of these findings and of extensive observations of patterns, direction of coiling and changes in the length of the chromonemata, a series of hypotheses concerning the coiling mechanism have been proposed.

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THE NITROGEN NUTRITION OF GREEN PLANTS. II[1]

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INTRODUCTION

Since the subject was last discussed in this journal (110) there have appeared eight reviews on protein metabolism in plants, including an excellent book by Chibnall (44). Particular attention may also be called to reviews by Vickery (163–165) that emphasize, in part, the rôle of amino acids, amides and organic acids. Protein synthesis in plants has also been fully discussed from a physiological viewpoint by the late Prof. Petrie (115, 117) and the nature of leaf proteins by Lugg (88). Other accounts of nitrogenous constituents in plants have been submitted by Wood (190) and McKee (93). Available also are a number of résumés on mineral nutrition that include discussions of relationships of nitrogen to other elements (10, 64, 75, 76, 117, 138, 145). The translocation of nitrogen in plants has been reviewed by Mason and Phillis (100) and the biochemistry of symbiotic nitrogen fixation by Wilson (188). Recapitulation of the subject matter in the reviews cited has been largely avoided in the following discussions which deal with the initial stages in ammonium and nitrate nutrition. There is also included a survey of experimental work on nitrogen nutrition as related to the vegetative and reproductive responses of plants, a background for which is to be found in the research of Kraus and Kraybill (83) and with reference to grains in reports by Russell (128, 129) and Bishop (18, 19).

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NITRATE REDUCTION AND ASSIMILATION IN ROOTS AND LEAVES

It was brought out in an earlier review (110) and since emphasized by Olsen and Whitehead (113) that different kinds of plants vary in the quantity of nitrate they store and in the organs that contain it and that where nitrate is the chief external source of nitrogen a fairly high concentration must often be maintained in plants or vegetative growth will be limited. The importance of nitrate concentration in new protein synthesis has also been indicated by Phillis and Mason (118). On the other hand, although nitrate is probably the most important source of nitrogen in most soils it is not an essential constituent of plants, for vigorous growth and abundant fruiting may be obtained with ammonium nutrition and with plants entirely or practically free of nitrate. Nitrate represents nutrient material not yet metabolized, a potential source of organic nitrogen.

That nitrate reduction and organic nitrogen synthesis may occur in the roots of plants is well known (110), and it is obvious that directly or indirectly this must involve a decrease in reserve carbohydrates or their derivatives. Robbins and Schmidt (124) found that excised tomato roots required no other source of nitrogen than nitrate, provided the nutrient medium contained sucrose, mineral salts and vitamin B, or thiazole. Likewise Postma (119), working two-day periods with excised roots of oats in a solution containing glucose, indicates that added nitrate was rapidly reduced in the roots and new protein synthesized, accompanying which there was vigorous respiration. Haas (65) reports that the roots of lemon and orange reduced nitrate. Burstrom (32) found that wheat roots assimilated nitrate and that manganese was essential in its reduction to nitrite and ammonium accompanied by oxidation of sugars and elaboration of organic compounds of nitrogen. He feels that his experiments support the hypothesis that manganese is in the cell plasma and that other cations may obstruct the assimilation of nitrate by displacing manganese from this attachment.

Virtanen and Arhimo (167) grew peas and oats in water culture with nitrate as the sole source of nitrogen, and in light or in darkness the presence of nitrite and hydroxylamine was detected in the roots. On the other hand, with nitrate-free cultures containing ammonium sulphate neither nitrite nor hydroxylamine was found. From this they conclude that not only nitrite but hydroxylamine may be formed in reduction of nitrate. They also report that in the absence of certain organic acids NH_2OH may be reduced to NH_3. Chibnall (44) has also suggested that the steps in nitrate reduction may be: nitrate, nitrite, hyponitrous acid, hydroxylamine and ammonia.

There can accordingly be no doubt that reduction of nitrate and new protein synthesis can occur in the roots of plants. In some species, particularly in perennials, it is often mainly if not entirely in the roots, while in others nitrate may be assimilated in leaves or stems (110) or, according to Engard (56) working with raspberry, in all living cells where the proper conditions for nitrate reduction exist. Elaboration of nitrate thus does not appear to be a function of special organs; in fact, even the storage leaves of the bulb of the onion apparently reduce nitrate, according to a recent report by Bennett (17).

It is obvious that in roots, or in other underground organs, the reaction occurs in darkness at the expense of carbohydrates or nitrogen-free derivatives. Because nitrate assimilation does proceed in the absence of light, is no reason, Burstrom feels (36), for concluding that there may not be cases where light is directly necessary for nitrate assimilation.

In the older literature (37) there is considerable qualitative evidence suggesting this. Burstrom (36), working only with wheat seedlings, presents the results of extensive quantitative trials from which he concludes that nitrate contained in the leaves of wheat was assimilated only in light and in an atmosphere containing carbon dioxide.

He grew wheat seedlings at essentially constant temperature under artificial light employing nitrogen-filled incandescent filament type lamps. When two leaves had partially emerged from the sheath the plants were cut off at the tip of the coleoptile, and roots and all tissue below the tips of the coleoptiles were discarded. Burstrom found that there was no material loss of nitrogen into culture solutions from the cut end of the seedling. The plant was placed in a glass tube with its severed basal end in solution, and the tube was connected with an apparatus for measuring CO_2 exchange. The plants in the tubes were then immersed in a water bath and maintained in all cases, for only 24 hours, at various light intensities or in darkness in normal CO_2-containing air or in air essentially lacking CO_2. Analyses are reported on an absolute amount basis, for nutrient solutions, for plants and for CO_2 of the atmosphere at the beginning and end of test periods.

His replicated trials were in good agreement and indicated that in light in the absence of nitrate practically all absorbed CO_2 was accounted for as hexoses and sucrose. When nitrate was present it was not assimilated in darkness, but in light with increasing intensity there was an increase in assimilation of nitrate and in intake of CO_2. The increased absorption of CO_2 over that of the nitrate-free culture could not be accounted for as sugars (the seedlings were essentially free of starch), and the intake of CO_2 over that used in synthesis of sugars was found to be proportional to the amount of assimilated nitrate. For each mol of assimilated nitrate there were apparently about 14 mols of CO_2 bound up into compounds of non-sugar character termed "CN assimilates".

In marked contrast to assimilation of nitrate the protein content of his ammonium-supplied cultures increased in darkness at the expense of carbohydrates. According to Burstrom, it would therefore seem to follow that nitrate in wheat leaves in the light was not reduced to ammonium but rather combined with undetermined CO_2 products prior to complete reduction.

Burstrom does not imply that the pattern for nitrate assimilation in the leaves of wheat seedlings applies to all genera. In fact, as already mentioned, there are many plants that rarely if ever contain nitrate in their aerial organs (110). This is emphasized by Burstrom in his recent review of nitrate nutrition (37). However, in harmony with Burstrom's results, Blackman and Templeman (22), working with grasses and clover grown in soil, found that shaded leaves apparently lost their ability to elaborate nitrate rather than ammonium ions to organic nitrogen. However, working with soils presumably containing nitrifying bacteria, much of the nitrogen supplied as ammonium probably was converted in the soil to nitrate. Also Rombeck (125), experimenting with *Helianthus* leaves in the light in an atmosphere lacking CO_2, found that nitrate reduction was curtailed more than might be expected on the basis of a comparatively small initial decrease in carbohydrate content of the leaves. Pearsall and Billimoria (114), working with bacteria-free cultures, floated segments of sterilized narcissus leaves on solutions containing ammonium nitrate and sucrose adjusted to pH 5.4. Gains in protein occurred only in the light with loss in total nitrogen, however. Contrary to Burstrom's results with wheat they report no measurable assimilation of ammonium in darkness. No losses were observed when organic sources of nitrogen were used. They explain the loss, although without direct chemical evidence, as owing to reduction of nitrate to nitrite and reaction with amino acids to form gaseous nitrogen. Seemingly in accord with the foregoing interpretation there is recorded by Lovell (87) evolution of extra oxygen in the light by *Elodea* leaves supplied with nitrate. Mothes (104), however, employing excised leaves of bean, maize and tobacco, did not obtain loss of nitrogen with assimilation of nitrate, except where he added nitrite to the culture solution. It would seem rather peculiar if loss of gaseous N should be found to be a usual phenomenon in intact non-senescent plants supplied with a complete nutrient solution. However, where Vickery *et al.* (166) grew *Narcissus* in sand culture in distilled water he found a loss in nitrogen of 15% of that present in the original bulbs, but exosmosis may occur (154) in distilled water cultures and may possibly have been a factor in the results obtained. An explanation of the phenomenon of loss of nitrogen must await further research. Nightingale and Robbins (110) reported only a slight loss in amount of nitrogen, probably not significant, in *Narcissus* bulbs grown in a nutrient solution complete except for nitrogen-containing salts.

In contrast to nitrate reduction there is evidence indicating that reduced forms of nitrogen may be oxidized to nitrate in the tissues of plants. *Narcissus* grown in distilled water or in a complete nutrient solution with nitrogen as ammonium sulphate was found by Vickery *et al.* (166) to contain traces of nitrate, even though the bulbs at the start of the experiment contained none. However, as they point out, the sand, although washed, was not bacteriologically sterile. On the other hand, their earlier work with excised leaves of tobacco (162) and rhubarb (160) in water culture in darkness demonstrated that there was a significant increase in quantity of nitrate. Also McKee and Lobb (94) found that nitrate increased in Swiss chard and in tomato leaves that were dried at room temperature. Likewise Pearsall and Billimoria (114) record an instance of nitrate synthesis in segments of narcissus leaves floated on nitrate-free culture solutions containing ammonium chloride. Thus nitrate synthesis, although not yet clearly demonstrated in experiments with intact plants, occurs at least in plant tissues undergoing autolysis.

Despite years of practical experience in agriculture and experimental evidence that does not support the conclusion, reports appear from time to time (131) that non-leguminous plants can fix atmospheric nitrogen. Ruben *et al.* (127), using radioactive N^{13} gas, obtained results with barley which they did not consider conclusive with reference to nitrogen fixation, their experiments being necessarily limited to exposure to N^{13} for not more than 20 minutes, as N^{13} has a half life of only 10.5 minutes. On the other hand, Burris (31) employed N^{15} which does not necessitate a time limitation. His plants, bacteria-free barley, bacteria-free clover and inoculated clover (*Rhizobium trifolium*), were grown in sterilized quartz sand in an atmosphere of 20% oxygen and 80% nitrogen, the N used containing 13.87 atoms % of the isotope N^{15} or 13.5% N^{15} excess, the normal quantity of N^{15} being 0.37%. After six weeks the plants were harvested, and no fixation of N^{15} by the bacteria-free barley or by the bacteria-free clover could be demonstrated, whereas, there was fixation by the inoculated clover. These results and the well established evidence of the respective nitrogen-depleting and nitrogen-replenishing capacity of non-leguminous and leguminous crops do not point to the conclusion that atmospheric nitrogen is fixed in significant quantities by bacteria-free plants.

AMMONIUM NUTRITION

Ammonium nutrition is no longer a matter of limited academic interest but of practical importance over many thousands of acres of arable land. Soil fumigants, such as D-D mixture consisting of 1 : 3 dichloropropene and 1 : 2 dichloropropane, are employed extensively to control nematodes and certain insect pests (39, 40). On the other hand, soil fumigation has been found to markedly increase plant responses in the field, even though the soil was apparently essentially free of undesirable organisms. This occurred where ammonium rather than nitrate was used as the source of nitrogenous fertilizer and was correlated with suppression of soil nitrifying bacteria. Plants in fumigated soils may thus be supplied largely with ammonium rather than nitrate for a period often of several weeks (150, 151). In contrast, where a fertilizer such as ammonium sulphate is used in an unfumigated soil it is of course usually converted to nitrate rapidly, and plants absorb little nitrogen as ammonium.

In a well buffered nutrient medium, provided there results no deficiency of essential elements, there is required no different pH value for ammonium than for nitrate nutrition. This has already been discussed in considerable detail along with pH ranges at which desired plant responses can most readily be attained (110, 169, 170). These conclusions have recently been confirmed by Burstrom (33) and by very careful studies of hydrogen-ion concentration by Arnon, Fratzke and Johnson (8) and Arnon and Johnson (9).

Where ammonium has been the major external source of nitrogen, as following soil fumigation coupled with the use of ammonium sulphate, or where water cultures have been employed, ammonium in contrast with nitrate has often been associated with relatively rapid synthesis of new protein in darkness or in light and with relatively rapid growth, as compared to nitrate-supplied plants, provided the carbohydrate reserve of the plant has been adequate and there has been no lack of essential mineral elements (110). In connection with these responses it may be significant that ammonium requires no reduction prior to its use in new protein synthesis, whereas nitrate, at least in underground organs or in darkness, must be reduced to ammonium, an energy-requiring reaction that is eliminated when ammonium is supplied directly.

However, where the light rates are often low, as in glass houses and under seasonal conditions of low sunshine, carbohydrates may become more of a limiting factor with ammonium than with nitrate nutrition (36, 140–142, 161, 168). While the foregoing results were based largely on analyses of vegetative organs, Demidenko and Barinova (53) found that spring wheat supplied with ammonium stored more protein but less starch in the grain than the cultures furnished with nitrate. Of particular interest perhaps in relation to Burstrom's work, Blackman and Templeman, in experiments with grasses and clovers (20, 21, 22), found that shaded leaves lost their ability to elaborate nitrate rather than ammonium ions into organic nitrogen. However, there were not included comparable studies of root systems, but their results with leaves seem in accord with Burstrom's (36) in that nitrate assimilation in wheat leaves, as already noted, was concluded by him to be a photosynthetic process, whereas light was not required for ammonium assimilation, the latter process taking place at the expense of stored carbohydrates.

While there is considerable evidence to indicate that characteristically ammonium nutrition tends to result in relatively rapid vegetative growth and a low-carbohydrate plant, this was not the case where ammonium and nitrate nutrition of *Narcissus* was compared (166), the nitrate-supplied plants being lower in carbohydrates. However, the plants of the ammonium cultures absorbed no more inorganic material than others in distilled water culture, while the plants with nitrate increased greatly in ash. The potassium, calcium and magnesium content of the nutrient solutions of the ammonium and nitrate cultures were the same. If the ammonium culture solutions had been materially increased in content of K, Ca and Mg, different responses might possibly have been obtained, for ammonium commonly drastically limits intake of other cations, as will be shown presently, sometimes even leading to excretion of them.

EFFECTS OF AMMONIUM AND NITRATE ON THE ABSORPTION OF OTHER ESSENTIAL IONS

In addition to typical influences on the carbohydrate content of the plant ammonium and nitrate have characteristic effects on absorption of other ions. For example, in pineapple fields (110) there is clear cut evidence to show that nitrate, directly or indirectly, repels the intake of phosphate. Fertilization with phosphate, if the plant is low in phosphorus, may increase fruit production, but if nitrate is simultaneously deficient, in relation to carbohydrate, added phosphate will often decrease the yield of fruit by two or three tons per acre. This is apparently because phosphate fertilization under such circumstances further limits the intake of needed nitrate. Contrariwise potassium and nitrate each favors the intake of the other. In fact, very high levels of nitrate fertilization may not result in important increases in nitrogen content of the pineapple plant unless accompanied by an adequate supply, particularly of potassium, but also of other cations. On the other hand, with soil fumigation and with ammonium rather than nitrate as the chief source of nitrogen, very much heavier applications of potassium- and other cation-containing fertilizers are indicated. Not only in the field but in water cultures, pineapple plants display comparable responses, as brought out by Sideris and Young (142).

Such observations are not, however, limited to pineapple. Anderssen and Bathurst (4), studying the nitrogen and phosphorus ratio in oranges, found that a high nitrogen content of fruit juice was correlated with a low phosphorus content and *vice versa,* and that a high plane of nitrogen fertilization limited phosphorus intake and was accompanied by a decrease in yield. The nitrogen-containing fertilizer employed was ammonium sulphate, but lacking soil fumigation there can be little question that essentially all nitrogen absorbed was as nitrate. Also working with citrus trees, Chapman, Liebeg and Vanselow report that in soil, sand or solution culture high levels of phosphate curtailed nitrate intake and were associated with early senescence of leaves, even though the trees received monthly additions of calcium nitrate, and later (41) that nitrate-deficient plants were relatively high in phosphorus and that potassium exerted a favorable influence on nitrate absorption.

Breon and Gillam (28) found that tomato plants supplied with nitrate and a low level of phosphate accumulated nitrate apparently due to relatively rapid absorption rather than limited elaboration. Apparently the rubber tree responds in like manner, as indicated by leaf-analyses reported by Chapman (43). Hamner (66) found that soybean plants with a nutrient solution deficient in nitrate but containing large amounts of phosphate exhibited phosphorus toxicity but that only a small quantity of additional nitrate counteracted what would otherwise have been a toxic concentration of phosphate. The peanut plant, as shown by Moore (103), seems also to fall in much the same category, being particularly sensitive to phosphate increases under conditions of nitrogen nutrition that are low.

Arnon (7) studied the mineral composition of barley with ammonium or nitrate nutrition and found that with ammonium the plants had a lower base content, but were higher in phosphate than plants furnished with nitrate. Arenz (5), Schropp and Arenz (133) grew a variety of crop plants including barley in soil, sand and water culture with ammonium or nitrate nutrition, and invariably found that relatively high potassium levels were needed for the ammonium cultures and where nitrate was the source of nitrogen a liberal quantity of phosphate. In some cases excretion of NH_4 from the ammonium-supplied plants was found that could be counteracted by increasing the potassium supply. Additional references might be cited, but the characteristic relationships of ammonium and nitrate to absorption of other ions are indicated. Of course, typical patterns may not always be displayed. Ions that are often present but undetermined such as chloride may limit nitrate absorption. Shear (136) has reported, as have many others, on the antagonistic effect of chloride on nitrate intake. He found that chloride supplied to tobacco plants at apparently non-toxic concentrations resulted in a lowered level of nitrogen nutrition. By furnishing other plants with less nitrate he obtained comparable responses without adding chloride.

As brought out in an earlier review (110), iron deficiency in plants, such as pineapple (141) and gardenia (30), is often more severe with nitrate than with ammonium nutrition. This appears to be associated with a relatively high degree of residual acidity which tends to keep iron in solution in typical ammonium sulphate–supplied cultures. Even with very rapid renewal of culture solutions and no marked change in the pH of the solution as a whole, films in contact with the absorbing roots of cultures supplied with nitrogen chiefly as ammonium are typically relatively low in pH as compared to others with nitrate as the major source of nitrogen (110, 169).

AMMONIUM AND NITRATE NUTRITION AS RELATED TO THE OXYGEN REQUIREMENTS OF THE ROOT SYSTEM

In further consideration of the relative merits of ammonium and nitrate as sources of nitrogen it should be recognized that the assimilation of ammonium, unlike that of nitrate, is increased by a relatively high oxygen supply in the nutrient medium and facilitated by the presence of a comparatively high amount of a heavy metal such as manganese or copper which act as oxygen catalysts. This has been demonstrated by work which includes experiments with soybeans, tomato and oats; other experiments with citrus trees (6, 75, 139, 61, 65) furnish further confirmation of the fact that nitrate cultures require far less oxygen than those lacking an external source of nitrogen or than those supplied with ammonium. In fact, an oxygen content of the nutrient substrate essential for ammonium nutrition or for a minus nitrogen culture may be so high as to be toxic if nitrate is the chief source of nitrogen. Apparently the oxygen evolved in nitrate reduction is utilized in the respiratory processes of the root cells.

While not peculiar to either the ammonium or nitrate ions it should be emphasized that absorption of salts by the root systems of plants is correlated with active respiration. For this not only an oxygen supply is essential but an adequate carbohydrate reserve in the root system, as demonstrated by Hoagland and Broyer (74) with barley roots. Also limiting the carbohydrate content of root systems of orange trees by girdling the stems was reported (60) to limit nitrogen absorption. While not associated alone with absorption it may be noted that Alten and Gottwick (1) found that increasing the nitrogen content of culture solutions gave an increase in respiration, as measured by the CO_2 excretion of the roots of maize.

NITROGEN AND CARBOHYDRATE RESERVES AS RELATED TO ROOT DEVELOPMENT

Carbohydrates are necessary not only for absorption but, of course, also for the carbon skeleton for organic nitrogenous compounds. The extent and character of root systems is also correlated with carbohydrate content and factors influencing it. Horticulturists generally have found a relatively strong development of root systems, with many fine lateral rootlets, associated with liberal carbohydrate reserves for such diverse plants as vegetables, cereals and trees. It was found by McElwee (91) that an external supply of sucrose enhanced the rooting of cuttings of a number of ornamental plants, and Burstrom (34, 35) emphasizes the apparent formative influence of carbohydrates on root growth. He employed cultures of isolated wheat roots. Where no glucose was added neither division nor elongation of cells occurred, but with increasing amounts up to a certain point both division and elongation increased. At higher concentrations of glucose the cell number did not change, but the cells increased in size transversely, with increase in diameter of the roots. This was not apparently connected with the osmotic concentration of the culture solution, but depended upon the quantity of glucose itself.

White (185, 186) reports that the length of the root of *Lemma minor* is a sensitive indicator of the carbohydrate and nitrogen content of the fronds, either a high light intensity or limited nitrogen supply or both being associated with relatively long roots and a high content of carbohydrates. Also in lettuce (191), peach (174), apple trees (23) and pine seedlings (102) typical responses were obtained, a low level of nitrogen nutrition being associated with the development of relatively extensive root systems.

SALT CONCENTRATION OF THE NUTRIENT SUBSTRATE

Another factor of importance in nitrogen nutrition is the salt concentration of the nutrient substrate. The work on effects of excessive amounts of salts, such as are formed in saline soils, has been ably reviewed by Magistad (96). It may be well to note, however, that high levels of calcium chloride or sodium chloride have been found to limit the absorption of nitrate (171, 173), a result to be expected on the basis of experimental results of others already cited. However, this was not associated with carbohydrate accumulation, presumably in part, because of chlorosis of the leaves (95).

On the other hand, increasing the osmotic pressure of nutrient solutions by uniformly increasing all constituents to a level approaching in effect that of physiological drouth has given a marked increase in nitrate content of plants but has curtailed new protein synthesis and been associated with carbohydrate accumulation. The net effect of such treatment is to give plant responses essentially similar to those that may be obtained with a moderate or low level of nitrogen nutrition (110). Burkhart and Biekart (30) found that for vigorous growth during the vegetative stage gardenia should have a relatively low concentration of nutrient solution, but in order to curtail vegetative growth and to obtain early blooms an increase in concentration of solution was desirable. They found that such manipulation gave them plants high in carbohydrates that bloomed relatively early, but that where the concentration of solution employed was too high for seasonal light conditions it gave typical effects of extreme nitrogen deficiency with abscission of blossoms and splitting of the corolla.

PHOTOPERIODISM

While light intensity is often a limiting factor it is well recognized that the photoperiod frequently dominates the responses of certain kinds of plants. Nevertheless, nitrogen nutrition under both long and short photoperiods is not without important influences on yields of vegetative and reproductive organs. Scully, Parker and Borthwick (135) grew different varieties of soybeans under a short photoperiod and they found that the position of the first flower did not vary with the nitrogen levels imposed. However, when grown with long photoperiods plants of certain varieties initiated their first flower primordia at higher nodes as the amount of nitrate in the nutrient solution was increased. The same workers (134), studying interactions of nitrogen supply as related to responses of onion under different photoperiods, found that plants grown with a photoperiod at or near that critical for bulb formation exhibited the greatest bulb development with the lowest nitrogen level tested and the least with the highest plane of nitrogen nutrition. In contrast, plants receiving a photoperiod substantially longer than that critical for bulb formation showed no difference in bulb development when supplied with different amounts of nitrogen. Brenner (27), using only one photoperiod for any one experiment but working in both field and greenhouse in all cases, obtained relatively early bulbing of onions by limiting the nitrogen supply.

In a report on nitrogen nutrition in relation to photoperiodism in *Xanthium pennsylvanicum,* Neidle (109) found that plants with either long or short photoperiods receiving nitrogen bloomed earlier and produced more fruits than those deprived of it. However, the results do not make clear what responses might have been obtained with an intermediate amount of nitrogen. Clearly much practical information has been developed through studies of the nitrogen nutrition of plants under different photoperiods, but clarification of interrelationships has yet to be attained. One of the more recent attempts in this direction is that of Sheard (137) who analyzed the stem apexes of tomato and of three varieties of chrysanthemum that had been grown under long and short photoperiods. Analyses were made at about weekly intervals, but the transition from vegetative to reproductive development took place without there being detected significant changes in determined carbohydrates and nitrogenous constituents.

CARBOHYDRATES, HORMONES AND NITROGEN

On the other hand, particularly in the case of apple trees, there is considerable evidence concerning factors that are closely correlated with blossom bud initiation. The excellent work of Harley, Magness, Masure, Fletcher and Degman (70) is of particular interest in this connection. They avoided employing samples for analysis of widely different morphological components but compared tissues that initiated blossom buds with tissues forming leaf buds. In their study they found that a high starch rather than a high nitrogen content appeared to be correlated with the inception of blossoms. A slightly lower concentration of reducing sugar was also found in differentiating buds. They suggest that energy was required for differentiation, that this was supplied by reserve carbohydrates which in the apple tree is in large part starch, and that the reducing sugar content was relatively low, being used by the differentiating spurs in the process of blossom-bud development. Their data would seem to indicate also that if a specific factor, or factors, is the active principle in the inception of flower primordia this factor or hormone was very intimately related to the synthesis and deposition of starch. They emphasize the close relationship between leaf area and blossom-bud initiation, and that much less leaf area was required for blossom buds to form if the movement of photosynthates to the roots was prevented by ringing. For example, in defoliation tests in the absence of fruit, a larger leaf area was required to form blossom buds on ungirdled than on girdled branches, indicating a prior demand, presumably by the roots for starch and other leaf-products essential for blossom-bud development. Greene (62) and Murneek (105) also have demonstrated that ringing or girdling will give an increase in starch content above the girdle, and will give a marked increase in size and number of fruits that set, particularly if the trees are high in nitrogen and vigorously vegetative.

In agricultural practice it is obviously important to avoid a deficiency of either nitrogen or carbohydrates as related to a desired vegetative or reproductive response. This is true not only of apple and other tree fruits (98, 106, 107, 120, 143, 187) but has been found to be significant in the culture of many species of plants (37), including seed production in biennials such as sugar beet as shown by Pultz (121). However, there is essentially no direct evidence available on plant composition as possibly related to specific blossom-forming substances, but in addition to results already cited the work of Stout (147) provides information. He took separate shoots of annual beets having more than one shoot and subjected them to different photoperiods. The parts exposed to short days remained vegetative, even though connected to parts exposed to long days. Shoots kept in darkness and connected to others under a long photoperiod developed seed stalks. Exposure of a shoot to short photoperiods was interpreted as apparently producing sufficient carbohydrates for that part, and hence none was translocated to it from the shoot exposed to a long day. Shoots in darkness, however, were said to have become dependent upon others upon the same plant exposed to long day conditions, and carbohydrates along with blossom-inducing materials were presumably translocated to the darkened shoot. These results, as in apple, suggest that hormones inducing blossoming may be translocated with carbohydrates.

That there is perhaps also an intimate relation between the same or similar hormones and carbohydrate requirements of roots seems suggested by observations already recorded and by the following reports. To cite only a single case of many similar experiments that might be mentioned, Stuart (148) treated cuttings of kidney bean seedlings with indoleacetic acid, and carbohydrates and other materials were translocated in quantity from the leaves to the hypocotyls where there was rapid swelling and profuse root production. Others (158), giving an account of experiments with hibiscus cuttings, show that an important function of leaves as related to root initiation and development is to supply the cutting with sugars, and McElwee (91), in tests on the rooting of cuttings of a number of ornamental plants, mentions that sucrose added to the nutrient substrate exerted as favorable an effect on root development as indoleacetic acid in certain species. Again it would seem to be suggested that carbohydrates, along with certain hormones, are intimately associated not only with flower bud differentiation but with root development as well.

NITROGEN AND CARBOHYDRATE DEFICIENCY

Cotton. Carbohydrates have been emphasized in the foregoing discussion, but that the supply of nitrogen available for new protein synthesis may be critical in relation to the carbohydrate content of plants and their capacity for reproduction and vegetative growth is shown by Wadleigh's work with cotton (172). He comments that in the field cotton plants may become too vegetative for optimum boll production, though inadequate yields also result if plants are small and stunted. His experimental plants were grown in sand culture at four levels of nitrogen supply—8, 25, 75 and 225 p.p.m.—all as nitrate. In general, the lower the nitrogen the higher the carbohydrate content. As related to the preceding discussions on roots it is of interest too that his relatively low carbohydrate plants with 225 p.p.m. of nitrogen in their nutrient solutions contained an absolute amount of N only slightly more than that of other cultures supplied with one third as much nitrate. This again would seem to emphasize the already stressed importance of carbohydrates as related to absorption and utilization of nitrogen, and supports other similar observations with cotton (52).

Wadleigh reports, further, that the average amount of seed cotton in order of nitrate in the nutrient solution was 27, 57, 120 and 143 grams per plant. Nitrogen deficiency was associated with curtailed yields. After a plant had set a sufficient number of bolls to deplete its nitrogenous reserves, all subsequently formed young bolls abscissed, later young squares were shed and ultimately even the terminal buds of the fruiting branches aborted. In the highest nitrogen cultures lack of an adequate carbohydrate reserve limited boll production to a lower level than would doubtless have followed if the light conditions of the whitewashed greenhouse where the plants were grown had not been about half that of light intensity outside. Nevertheless the highest average yield per plant was several times better than that of the average field-grown plant in Arkansas.

Wadleigh also provides information showing that the development of a vigorously fruitful cotton plant entailed numerous channels of carbohydrate utilization in addition to that needed for absorption and assimilation of nitrogen. Yet without an adequate content of elaborated nitrogen sugars condensed to starch which accumulated unused, particularly in his lowest nitrate group of plants. Metabolic conditions conducive to condensation of sugars should have been favorable for cellulose formation, but such a tendency could scarcely be expressed in the desired development of cotton (seed fibers of cellulose) in the lack of elaborated nitrogenous materials essential for the initiation and development of ovules. It is of interest also that cotton seed oil, derived from sugar, reflected the status of carbohydrate reserves in the plants. At higher nitrate levels with lower carbohydrate content there was an increase in protein and a decrease in the oil contained in the seeds. Likewise the content of gossypol, with its primary precursor, the glucose molecule, was closely correlated with the carbohydrate content of the plant.

Eaton and Rigler (55) also give an account of experiments with cotton grown in the winter in the greenhouse with plants under muslin shades at a midday light intensity of about 1,000 foot candles.

Other experiments were conducted during long summer days outdoors with a midday light rate of about 10,000 foot candles. In both experiments it appeared that limited carbohydrate reserves were associated with shedding of bolls. However, they make the significant observation that carbohydrate concentrations were not reduced in summer to the levels found in the winter-grown plants before abscission of bolls occurred. This is interpreted as indicating that the high light conditions encountered in summer may have in some way depressed carbohydrate utilization. While these responses may well be associated with other than the usually recognized phases of nutrition, a factor markedly influencing utilization of carbohydrate reserves is the content of mobile elaborated nitrogen. This is not made apparent in their analyses limited to total nitrogen, but the content of amino acids and related compounds is shown by Wadleigh to be critical in cotton for maintenance of ovules and to prevent shedding of bolls. Nevertheless in general at the lower concentrations of nitrate employed their results for the summer months and those of Wadleigh seem in fair agreement. But their highest nitrate cultures contained 396 p.p.m. of nitrogen as nitrate, and the nitrogen content of the leachates were up to over double this value, which would be expected to limit rather than accelerate new synthesis of amino acids, related compounds and protein, especially under high light conditions, as brought out in discussions of effects of salt concentration in this and in an earlier review (110).

In harmony with the foregoing Dunlop (54) reports that conditions unfavorable for synthesis of sugars, such as cloudy weather, artificial shade, close spacing of plants or a reduction in number of hours of sunlight, increased abscission of cotton bolls. Also under these conditions gains in fruiting were not usually obtained from nitrogen fertilization, but nitrogen added to soil in pots gave striking increases in fruiting in the summer months when conditions were relatively favorable for manufacture of carbohydrates.

Cooil's (51) studies with *Cucuricta pepo* are not dissimilar, and while his analyses and other records presented have not always distinguished between the cause and the effect of plant responses as related to chemical composition, there can be little question as to their practical implication in nitrogen nutrition. Dark green strongly vegetative plants that developed during a period of cloudy weather were included in his experiments. They were high in nitrate and nitrate-free nitrogen but low in carbohydrates and unfruitful. Less green but vigorous fruiting plants were lower in nitrate and organic nitrogen but higher in carbohydrates. There were also described plants of a third metabolic category that were even higher in carbohydrates but lowest in organic nitrogen and lacking in nitrate. These plants had comparatively yellow foliage and produced blossoms, but they all abscissed, a response apparently much the same as Wadleigh obtained with cotton. Likewise Moore (103) grew peanuts in sand culture with various levels of nitrogen and obtained plants of carbohydrate and nitrogen content and with growth responses that followed a pattern similar to that already recorded for other species. Beckenback, Robbins and Shive (16) report that increasingly high amounts of nitrate in complete nutrient solutions increased the nitrate and elaborated nitrogen in the tissues of the vegetative organs of the corn plant with a decrease in content of sugars, starch and hemicelluloses. Working with barley leaves, Gregory and Sen (64) found that nitrogen deficiency led to much increased sugar, much reduced protein and amino nitrogen and a low respiration rate. Directly or indirectly respiration appeared to be positively correlated with amino nitrogen, possibly a reflection of overall effects of a liberal nitrogen supply on cell protoplasm. Although single experiments do not appear on the surface to be always in agreement, it would seem that respiration rate may well be influenced by two opposing factors. In low-nitrogen plants, for example, lack of protein may limit enzymatic activity, but, on the other hand, the high concentration of carbohydrates may accelerate it. There has been discussed in several recent reviews (44, 115, 117) the matter of respiration as related to the nitrogen and carbohydrate content of plants, and of proteolysis that commonly increases as carbohydrates decrease in mature organs. Even though there may be no inorganic nitrogen available this often leads to increased growth until carbohydrates become limiting, as apparently in shading or pruning plants, or shifting them to continual darkness (110). It would be of interest to know in this connection what the rôle of growth substances may be as associated with such responses. Avery (11–13) finds that auxin is relatively high in tissues well supplied with nitrogen. While the subject of protein metabolism, as already mentioned, is not considered in detail in these discussions, a distinctly new finding by several investigators (73,

162) concerning it should be noted. They employed isotopic nitrogen in nutrient solutions and by this means found that there is a continuous chemical interaction between the elaborated nitrogen of tissues and that of the nutrient. Results analogous to these have also been observed in the tissue proteins of mature animals (123).

Temperature is an environmental factor, in addition to nitrogen supply, salt concentration and light, that may have marked influences on the carbohydrate content of plants and their responses to nitrogen. Generally speaking, low temperatures favor carbohydrate accumulation, other factors being equal, as shown with tomato (58) and in comprehensive studies of effects of temperature on the carbohydrate content of that plant under controlled conditions in the greenhouse and recorded temperature conditions in the field (180–184). Concerning effects of low temperatures on intake and elaboration of nitrogen it is noteworthy that Batjer, Magness and Regeimbal (14, 15) find that the roots of dormant apple trees, though sometimes only after a lag of four weeks, can absorb and synthesize new protein from either nitrate or ammonium with temperatures of the nutrient medium as low as 32° F. Their data indicate that there was little if any translocation of nitrogen from the roots while the tops were still dormant. Absorption and elaboration of nitrogen during winter months apparently contribute in quantity to nitrogen requirements of the growing tops. Others (97, 106) find in mature orchards that nitrogen is absorbed by apple roots in the fall and winter and held there until growth starts in the spring.

Grains and Forage Crops. Turning to studies of the nitrogen nutrition of grains, reports on which for the most part have appeared prior to the period covered by this review, it seems apparent that under field conditions influences of climate are often dominant. Nevertheless there is apparent a fairly clear pattern of responses. Results of experiments with wheat up to 1932 are brought together and coordinated in an excellent review by Kraybill (84). The protein content of wheat is apparently dependent upon the amounts of carbohydrate and nitrogenous compounds available in the vegetative organs for the developing grain during the period of translocation. Environmental influences favoring a low protein–high carbohydrate wheat appear to include such factors as low temperature, long periods of growth with abundant sunshine after heading time, and heavy rainfall with leaching of nitrogen from the soil. Conditions conducive to a relatively greater abundance of protein in relation to carbohydrates may include high temperatures, short periods for maturation after heading, low light rate, moderate rainfall and an abundant supply of available nitrogen (*cf.* 78).

As emphasized, the composition and yield of grain is determined by climatic factors as by level of available nitrogen in the soil. As is well known, effects of nitrogen on the quality and quantity of grain vary markedly with the crops of different years, even in the same location. This is of course in contrast to pronounced differences in responses to nitrogen owing to variety (3, 77, 108, 132). Generally speaking, however, it has been found in nutrient culture work that a limited supply of available nitrogen consistently gives low total yields of grain but large individual kernels high in starch and low in protein (53, 101, 130). On the other hand, carbohydrate deficiency and excess of protein, particularly undesirable in barley, as related to malting quality, were found (79) to occur when late applications of nitrogen were employed. There resulted not only grain of poor quality but less of it. When applied early liberal applications of nitrogen have not always given typical effects of carbohydrate deficiency and protein excess where the nitrogen was utilized in large part in increased leaf development with presumably enhancement of carbohydrate content prior to heading. However, if the supply of nitrogen furnished early is excessive it may lead to lodging, as Watson (177, 178) shows, though, as he emphasizes, results from a given schedule of fertilizer treatments vary from year to year. He stresses particularly the importance of climatic conditions after ear emergence and through experiments employing black cardboard tubes for shading demonstrates that about 25% of the final dry weight of the ear is accounted for by the photosynthetic activity of the ear itself.

Discussions of anatomy, as related to lodging, can not conveniently be included in this review, but there is ample evidence in the literature (110) that plants continually deficient in carbohydrates have relatively thin cell walls and limited mechanical development, a situation conducive to lodging. An account of the anatomical responses of tomato stems to variation in supply of nitrate and other anions is given by Lyon and Garcia (89) who found that lack of nitrate was associated with limited stem diameter and limited development of both primary and secondary elements. There were not included plants of a carbohydrate deficient type, and within the limits of their experimental conditions stem diameter and cross sectional areas of the various tissues increased with increasing increments of nitrate, except where the supply of other essential cations was limited.

The responses of forage crops, as in the case of grains and in much the same way, are often dominated by weather conditions. But grazing practices or time and frequency of cutting along with any nitrogenous fertilizer employed impose additional influences that can markedly modify nutritive value of herbage, its palatability, botanical composition and longevity of the plants producing it. Harrison (71) found that too frequent defoliation of bluegrass, *Poa pratensis*, under comparatively low light conditions led to exhaustion of carbohydrate reserves of the rhizomes and root system, although the grass produced was apparently of a succulent palatable character high in nutritive value owing to its high nitrogen content. However, with depletion of carbohydrates, there was finally decreased production of blades and there followed death of the plants. This sequence of events was greatly accelerated by a high nitrogen supply, its absorption and elaboration being at the expense of stored carbohydrates in the underground organs. Apparently in accord with the work of Harrison, Blackman and Templeman (20–22) demonstrate that under a relatively high light rate addition of nitrogen markedly increased leaf production, even of frequently defoliated grasses (*Agrostis tenisis* and *Festuca risbra*), yet at lower light intensities the same fertilizer and defoliation treatments depressed leaf growth.

The season of application of nitrogen must also often be critical and for best results must doubtless be varied greatly with locality and kind of plant as related to a desired response. McCarty (90) has found in mountain brome, for example, that in early spring growth is at the expense of carbohydrates stored in the root and stem bases. The amount of carbohydrates in storage relate in large part to climatic conditions and other factors prevalent the previous autumn, which in turn must determine tolerance to early grazing and, presumably where economic, the advisability of applying or withholding nitrogen. Early grazing, when excessive in relation to the status of the plants employed, was reported by McCarty to have led to depletion of carbohydrates in the rhizomes and to have reduced plant vigor.

Working with another plant but with results in principle not unlike others described, Amos and Wood (2) point out not only that nitrogen additions under two different light intensities decreased the concentration of carbohydrates in rye grass, *Lolium sisbulatum*, with increase in elaborated nitrogen, but that when the water content of the leaves was reduced to a low level essentially no change in total carbohydrates occurred. However, effects of drouth on the chemical components of the plant, as shown by Clements (45, 46), may be expected to vary, depending in part upon constituents already present in the tissues at the time drouth is encountered and upon other factors (82). Sprague and Graber (146), however, working with alfalfa, *Medico sativa,* and with blue grass, *Poa pratensis,* with results in accord with many others, show that while nitrogen fertilization increased the total water used, the water requirement per unit of dry matter was decreased.

There would seem to be little doubt that nitrogen fertilization, if any, and cutting or grazing practices should not follow a rigid schedule but should be adjusted in part as indicated by carbohydrate and nitrogenous reserves, readily determined and often reflected with a fair degree of accuracy in the external appearance of the plant; and the well recognized fact that young succulent herbage, usually most desired, is richer in protein than in older plants of the same species and contains carbohydrates that are more digestible. There have been recent reports on protein and carbohydrate fractions of certain forage plants (92, 149, 179).

Tree Fruits. Chemical analyses of leaves have frequently been employed as an index of nitrogen requirements (68, 69, 157), the diagnostic value of which has been discussed by Ulrich (157) and Chapman (43). Under irrigation and where sunlight is seldom a limiting factor for any length of time and where there is relatively little variation in climatic conditions from site to site, considerable success can apparently be had where determinations are limited to total nitrogen, as in leaf blades of grape fruit (80) or nitrate in the petioles of grapes (156). However, in studies of citrus trees under the climatic conditions encountered Chapman and Liebeg (41, 42) noted that there were fluctuations in the dry weight yield of plants and plant parts of nearly identical nitrogen content. They come to the conclusion, with which the writer concurs, that the nitrogen percentage can be relied upon to indicate nitrogen requirements in a general way, but it is by no means exact.

Wark (176), in tests of plant tissues for use as a possible index of nutrient needs, worked with several species of plants, and in accord with the foregoing notes that in dull weather even with no more nitrogen added to the soil the content in the plants was higher than during periods of bright sunshine. Also Thomas and Mack (152) grew tomato plants in soil in the spring and fall in a greenhouse. They obtained nearly double the yields in the spring as compared to fall, but the nitrogen content of leaves for any particular plot in the fall was always much above that of the comparable plot in the spring. Admittedly there were included here two extremes in climatic conditions where the dominant factor controlling production was doubtless sunlight or opportunity for carbohydrate synthesis, as brought out by the same authors in another paper (153). But it would seem apparent that nitrogen content of plant tissues used alone as a guide to nitrogen fertilization is perhaps limited in usefulness largely to locations that vary little in climate.

Considerable work has been done on the nitrogen content of leaves of apple and other plants that has been reviewed by Ulrich (157). It shows quite conclusively that nitrogen fertilization is usually reflected in the leaves or, when leaves are not present, in other plant parts, as already brought out. Much of the work with apple trees has included attempts to determine effects of nitrogen on quality and yields of fruit as related to the percentage of nitrogen in leaf blades. Smock and Boynton (144) give results that indicate rather consistently that as leaf nitrogen increased under the climatic and cultural conditions prevailing, there was decreased firmness of the fruits and retardation of development of yellow ground color, and where significant differences in soluble solids occurred (Brix), they were less at the higher leaf-nitrogen levels. Boynton and Cain (26), through leaf analyses, indicate that in most of the orchards which they studied a high content of nitrogen in leaves was correlated with best yields but poorest fruit color, low nitrogen in general showing opposite trends. But there were orchards at both high and low levels of leaf-nitrogen that gave aberrant responses. Leaf-color estimates, included along with nitrogen analyses, seemed in general to show an increase in greenness with increasing nitrogen values. Magness, Batjer, and Regiembal (98) obtained results that were much the same with respect to fruit color and leaf-nitrogen but emphasize that similar fruit color would not always be expected at similar nitrogen levels under different conditions of light and temperature; and Wander (175), who obtained much the same results, notes that the size of the crop carried by the trees also influenced the nitrogen content, the leaves of trees with little or no crop containing larger amounts.

Effects of nitrogen nutrition in altering the fall coloration and maturity of grapefruit is reported by Martin (99). Fruit from trees of low nitrogen content in the fall developed yellow color much earlier than fruit from trees of high nitrogen composition. The fruit was sweeter, as measured by the Brix acid ratio of the juice when the nitrogen supply was limited in the summer months by competing cover crops. On the other hand, when nitrogen deficiency was severe, inducing senescence of the leaves and limited carbohydrate accumulation, there was almost complete crop failure. Cameron and Compton (38) find that although the nitrogen content of the leaves of grapefruit is at a maximum in the spring and minimum in midsummer, about half the total nitrogen of the tree is generally to be found in the leaves. Others (57, 80, 81) have shown that various fertilizer and cultural practices employed at different times of year, to obtain desired quality and good yields under climatic conditions prevalent in Arizona with water supplied mainly by irrigation, can advantageously be related to the nitrogen content of the leaves. Foliar analyses were in accord with Martin's work in showing that fruits from trees relatively low in leaf-nitrogen content were highest in quality and had notably thin rinds, development of which was closely dependent upon nitrogen during early stages of growth of the fruit. As might be anticipated, highest yields were obtained not with highest nor with lowest but with intermediate leaf-nitrogen values. They further found, as did others (189), that high ascorbic acid in grapefruit juice was associated with a low nitrogen content in the juice and an inverse nitrogen ascorbic acid relationship for various vegetables, while still others (67, 122) find correlation between light intensity and ascorbic acid, high light rates favoring its accumulation.

The carbohydrate and nitrogen content of the strawberry plant has been studied by Long and Murneek under conditions prevailing in Missouri (86). They find that the leaves in autumn may contain 70% of the total nitrogen of the plant, but during senescence of the leaves nitrogen content decreases rapidly with a simultaneous increase in the roots and stems. In the spring the process is reversed. Carbohydrate reserves follow a translocation pattern somewhat similar to nitrogen. From the standpoint of nutrition the practical indications were to fertilize with a view to building up adequate nitrogen reserves prior to fall. Spring applications were beneficial only in case of low nitrogen–high carbohydrate plants but comparatively ineffective if the plant entered the winter with a liberal nitrogen reserve. Data of the type presented, following correlation with yields under various environmental conditions, should make possible the ready determination of nitrogen requirements at any time on the basis of carbohydrate and nitrogen content of the Aroma strawberry plant, the variety tested.

Sugar Beets. Studies over many years in Europe and recent work by Gardner and Robertson (60) on effects of nitrogenous fertilizers on sugar beets have led to the general conclusion that over-fertilization with nitrogen results in a relatively low percentage of sugar in the storage roots, delays maturation of them and is associated with excessive leaf growth. On the other hand, if the external supply of nitrogen is too greatly limited low yields of beets will occur. Ulrich (155) has studied extensively the culture of beets both in pots and under field conditions in California and comes to much the same conclusions as European workers, neither an excess nor a deficiency of nitrogen being desirable for efficient production of sugar. But very significantly he finds that all beets high in nitrogen were not low in sugar nor that beets low in nitrogen were always high in sugar. Under some environmental conditions high nitrogen content was associated with high sugar and low nitrogen with low sugar. He points out that conditions favoring storage of sugar by high nitrogen beets include intense sunlight and warm days but cool nights, conditions conducive to rapid synthesis of sugars and limited loss of them by respiration. Beets low in nitrogen under these environmental conditions but not so deficient as to be senescent would be very high in content of sugar. With high day and night temperatures, even with high light rates, respiration, particularly when coupled with heavy nitrogen fertilization and rapid development of new tissue, may be so rapid as to result in storage roots with an extremely low percentage of sugar. These factors doubtless account in large part for the "high and low sugar years" which occur. Ulrich has employed the nitrate content of the outside petioles of sugar beet in field experiments and finds that in a given area at any one time whenever nitrate was high the percentage of sugar in the beets was lower than in beets that contained less nitrate in their petioles. It would seem that by employing sugar content of the storage roots of beet as the primary index and by controlling nitrogen supply to give suitable nitrate reserves in the petioles for the stage of development of the crop, much might be done to adjust the program of nitrogen fertilization to the requirements of the plant that vary with weather conditions in different locations and with variations in climate from year to year in the same location.

Sugar Cane. Although sugar-cane plant apparently does not store nitrate it is in other respects remarkably similar to sugar beet as far as nitrogen nutrition is concerned. Borden (24, 25) finds that in environments where sunlight is low, decreased yields of sugar resulted if the nitrogen applications were too high. Brink and Van Der Honert (29) point out that rich silty soil and soils liberally fertilized with nitrogen gave a high total yield of cane of low sucrose content. Rosenfield (126), working with cane plants in Egypt, records a marked depressing effect of heavy nitrogen fertilization on percentage of sucrose. In another respect nitrogen supply seems also to be significant in the production of sucrose in the cane plant, for Hartt (72) indicates in work done in part with detached leaves that when nitrogen deficiency was severe or when the nitrate supply was high there was diminution in the ability of the plant to convert hexoses to sucrose. This was not, however, the case when detached leaves were supplied with ammonium sulphate which did not seem to interfere with synthesis of sucrose. However, nitrate is not a commonly found constituent of cane leaves, whereas ammonium may be present in small amounts.

Clements (47–50) also has found that climate has a dominant effect upon yields and quality of sugar cane. He has compared locations where soil moisture and soil nutrients were essentially the same and correlated marked differences in yield with differences in sunlight. In the Hawaiian Islands there are differences in atmospheric conditions in different locations and from year to year in the same site that are important as related to plant responses. Clements, in integrating the growth of cane with unpredictable weather, has employed as his primary index the total sugar content of the sheaths of the young leaves. When sugar in this plant part is higher than the standard objective for the age of the plant, growth is slow. Factors associated with this are most commonly low nitrogen content of the leaf sheaths and low temperatures or low moisture. In contrast when sugar content is below the standard desired for the stage of growth the plant produces more growth than can be reconciled with the commercial objective maximum possible yield of sugar per acre for the year and location in question. Deficiency of sugar and excessive vegetative growth are frequently associated with excessive nitrogen or moisture or cloudiness and high temperatures.

Pineapple. Work with the pineapple plant (111), exactly the same in principle as that of Clements' with sugar cane, has made apparent that even in the same location the amount of nitrogenous fertilizer required may vary by as much as 75% in different years. The pineapple plant stores nitrate, and analyses of the leaves made for this nutrient material constitute a decidedly secondary index; determinations made at intervals as the plant developed show whether the nitrate reserve is increasing or decreasing, but nitrate values furnish little information of consequence for determining nitrogen requirements beyond this and excepting its relation to other essential ions (112). The primary index, reflected in leaf-color, is the starch content of the plant, the major carbohydrate reserve in the vegetative organs, and just as in case of sugar in the cane plant, if starch is too high as related to standards developed by relationship to yields in many field experiments, more nitrogen is known to be needed. If starch is low for the established values for the stage of growth, added nitrogen will not increase yields of fruit, even though the percentage of nitrate in the leaves approaches zero.

SUMMARY

Nitrate reduction may occur in roots, in storage organs and in stems and leaves. At least in underground organs nitrate reduction occurs in darkness with oxidation of carbohydrates or their derivatives. Burstrom (36) concludes that in wheat leaves nitrate reduction is a photosynthetic process that does not occur in the absence of light, although ammonium was elaborated in darkness in the leaves of wheat.

If a liberal supply of oxygen is available in the nutrient substrate, and of cations, including particularly potassium, and if there is adequate absorption of other essential elements, then ammonium is more rapidly utilized by plants than nitrate. However, ammonium nutrition typically results in relatively rapid depletion of carbohydrate reserves if conditions are not favorable for carbohydrate accumulation.

It is pointed out that environmental influences, such as low temperature or a high concentration of salts in the nutrient substrate, including even a high content of nitrogen, may lead to carbohydrate accumulation and deficiency of elaborated nitrogen in the plant.

Examples are given of experimental results apparently pointing to an intimate relation between carbohydrates and specific factors or hormones associated with the inception of flower primordia and root development.

Effects on various crop plants of nitrogen or carbohydrate deficiency are discussed along with methods by which the plant may be employed as guide in nitrogen fertilization using carbohydrate content as the primary index.

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STATISTICAL ECOLOGY. II.—A REASSESSMENT

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INTRODUCTION

Eleven years ago the present writer published in this journal an article on statistical ecology (1). The purpose of the present article is to review significant contributions to the subject since that time. This might be done in either of two ways: by making an annotated survey of the literature on statistical ecology published since 1936, or by re-examining, in the light of present knowledge, the value of statistical methods in the study of plant communities. In this article the second of these two ways has been chosen.

The first aim of the ecologist, as of any other scientist, is to describe the objects of his study quantitatively and objectively. A further aim is to include as many of the objects as possible in generalisations, to deduce the "type" from individual examples of the "type". In pursuance of these aims, some ecologists have devised statistical methods to describe communities, and they have tried to use these methods as a basis for classifying communities as associations. Eleven years ago it seemed reasonable to conclude that these statistical methods could not be used as a basis for classification, although they could be applied profitably in other ways. Is this conclusion still reasonable? Is the classification of a moorland, like the classification of a flowering plant, still a matter for subjective judgment? And has statistical analysis disclosed important facts about plant distribution which could not have been disclosed otherwise? The present article discusses, even if it does not clearly answer, these questions.

NATURE OF THE DATA IN STATISTICAL ECOLOGY

As soon as one proceeds from the mere inspection of a plant community to its analysis, it is necessary to focus attention upon one species at a time. The distribution of each species can be summarized by four characteristics:

(*a*). Fidelity: occurrence or non-occurrence of the species in the community;

(*b*). Abundance: the number of individuals of the species per unit area;

(*c*). Cover: the proportion of area covered by the species;

(*d*). Frequency: the chance of finding the species and its mode of dispersion.

Each of these four characteristics can be measured objectively and expressed quantitatively, and qualitative studies of them are, or ought to be, as out-of-date as qualitative studies of respiration. There is adequate proof that the older subjective methods are unreliable. West (27) has shown that estimates by different observers of percentage cover in pastures are subject to wide discrepancies. Hope-Simpson (12) has shown that the familiar estimates of abundance ("dominant", "abundant", "frequent", "occasional", "rare", *etc.*) are very unreliable, owing to heterogeneity among areas chosen as representative of the community, and to changes in the appearance of the community from season to season. Smith (25) has shown that a party of eight men, all familiar with the concept of density, and all with practical experience in the field, vary from 71.24% to 139.81% of the group average in their estimates of density of the same species over the same area. Over 40% of their estimates departed from the mean by more than 10%. Even after a week of intensive training, "high" estimators were finding range plants to have twice the density found by "low" estimators. There is no doubt, therefore, that statistical methods are required to give precise information about fidelity, abundance, cover and frequency.

ATTEMPTS TO ANALYSE PLANT COMMUNITIES OBJECTIVELY

Quantitative methods have not been of much help in the central problem of plant sociology: the extrapolation from the concrete community to the abstract association. When the ecologist stops his car and decides he has reached a "suitable place" for throwing quadrats on a community, he has already performed the major act of classification and he has performed it subjectively. Any subsequent quantitative analysis only elaborates, and possibly obscures, the original subjective decision. It is for this simple reason that the work of Scandinavian ecologists 25 years ago failed to reveal anything new about the status of associations: they had already settled the status before they laid a quadrat. It is still necessary, however, to sample communities in order to secure precise information about their composition; and therefore it is desirable to know what constitutes an adequate sample of a community.

In recent papers Cain (6, 7) has attempted to redefine an adequate sample plot, especially for alpine vegetation in Wyoming (7). He points out that the location of a sample plot is "partially subjective . . . it should be placed well within the community in a position that seems to be as nearly typical or average as can be judged". And the size of the sample plot "must be at least as large as the minimal area of the community type".

The minimal area, which Cain defines as "the least area upon which a community can develop its typical composition and structure", has played a notorious part in statistical ecology. Its attraction lies in the almost horizontal flattening of the species-area curve at a surprisingly low area: *e.g.*, 50 square inches in an English pasture; 40 square metres in an Australian desert; 32 square metres in an American alpine fell. The assumption commonly made is that the number of species is related to the area sampled by a curve of the form

$$n_a = n_m\ (1 - e^{-ka})$$

where n_a = the number of species on an area a, and n_m = the maximum number of species in the community. Cain draws attention to the fact that the point of apparent flattening of the curve, which is the point selected as the minimal area, depends on the scales used for ordinates and abscissae in the graph; he proposes to overcome this source of error by selecting for the minimal area that point on the abscissa where "the increment in number of species added per unit increment of area is equal to the maximum number of species found, divided by the total area sampled". This procedure adds to the complexity of the analysis without increasing its precision, for it assumes a knowledge of the shape of the extrapolated species-area curve, and there is no justification for such an assumption. The differential coefficient of the species-area curve, one value of which Cain proposes as a measure for minimal area, is dependent on the asymptotic value of the curve, which can only be guessed. Cain's method for selecting minimal area is not, therefore, really objective. It seems fair to conclude that Cain's studies confirm those of earlier workers in demonstrating the obstacles which lie in the way of using quantitative methods as a basis for the description of plant communities. Even the minimal area should still be selected subjectively on the basis of the observer's field experience.

Cain's attempt to define an adequate sample area brings into relief the limitations of the statistical method in plant sociology. The first limitation is that samples must be taken at random (even the method of composing larger quadrats from smaller ones, which Cain uses, is not free from objection). The second limitation is that the objects sampled must be approximately randomly distributed themselves, or in randomly distributed groups. In the study of plant communities the ecologist is obliged to transgress both these limitations. Even the micro-climate and soil of a plant community are not (except by a coincidence) randomly distributed. Having regard to the present state of our technique it is safe to predict that the classification of communities, like the classification of species, will be based on subjective criteria for a long time to come.

ANALYSIS OF THE DISTRIBUTION OF INDIVIDUAL SPECIES

Although attempts to classify communities objectively are disappointing, attempts to analyse the distribution of individual species are promising. During the last 11 years methods of analysis have been improved and good progress has been made toward putting this branch of plant sociology on a firm basis.

Advances in technique. The situation in 1936 was that estimations of the frequency of a species, although the most convenient way to sample its distribution, were unreliable unless the species was randomly distributed; and it had been shown that many species are in fact not randomly distributed. However, Blackman (5) has shown for some Swiss and English pastures that even where there is a notable departure from randomness the percentage frequency may provide quite a good measure of density. By using small enough quadrats Blackman obtained linear regressions between the logarithm of percentage absence for a species and its density. The slopes of the regressions did not as a rule coincide with the slopes calculated on the assumption of random distribution: there were as a rule too many empty quadrats at higher densities. This indicates, of course, that individuals are over-dispersed (contagiously distributed, as Pólya (20) has called over-dispersion). Despite this over-dispersion the percentage absence, suitably corrected, can be used as a measure of density. Blackman has accordingly put into the hands of ecologists a useful tool for following the changes in composition of small homogeneous plots. Blackman worked on areas of a few hundred square yards, and unpublished work indicates that the linear regressions he obtained can not be obtained when data are collected from larger areas. He found two kinds of divergence from Poisson distributions among the species he studied: (*i*) skewness without evidence of non-random dispersion: the Chi square values being high, but the relative variance remaining about unity; and (*ii*) some skewness accompanied by non-random distribution: the relative variance being significantly higher than unity, even though Chi square values are not very high. The first kind of divergence may indicate that individuals are randomly distributed within groups which are not randomly distributed; the second kind of divergence may indicate that individual plants are over-dispersed, though they may be in groups which are randomly distributed. Further studies are needed of these two kinds of divergence.

Clearly the study of over-dispersion is of major importance in statistical ecology, for recent work (8, 19, 22, 23) indicates that it is the rule rather than the exception in the distribution of plants, even over small areas. Recently a notable contribution to this study has been made by Cole (9). He points out that contagious distribution, *i.e.*, over-dispersion, is the rule rather than the exception among animals, for individuals are commonly associated in groups: in nests, or in holes, or as mating couples. It may be assumed, however, that over restricted areas the groups are randomly distributed. Making this assumption, Cole shows how a population of individuals may be analysed into n_g groups, consisting of n_1 single individuals, n_2 pairs, n_3 groups of three, and so on. Then:

$$n_g = n_1 + n_2 + n_3 + \ldots$$

If the mean number of groups per sample is m_g, this is the sum of the mean number of single individuals (m_1), plus the mean number of pairs (m_2), plus the mean number of groups of three (m_3), *etc.*: and:

$$m_g = m_1 + m_2 + m_3 + \ldots = \frac{n_g}{N}$$

where N = the total number of samples collected. If each group is randomly distributed, then the distribution of each group will follow its own Poisson series. The chance (N_0) of finding a sample without any groups at all in it will be the product of the zero terms of the several Poisson series, *i.e.*:

$$N_0 = N\,(e^{-m_1} \cdot e^{-m_2} \cdot e^{-m_3} \ldots) = N\,e^{-m_g}$$

From this foundation it is easy to express the chances of finding:

1 individual in a sample: $N_1 = N\,(m_1 e^{-m_1} \cdot e^{-m_2} \cdot e^{-m_3} \ldots) = m_1 N_0$
2 individuals " " " : $N_2 = N_0\,(m_2 + m_1^2/2!)$
3 " " " " : $N_3 = N_0\,(m_3 + m_1 m_2 + m_1^3/3!)$

and so on.

Cole applied this technique to the analysis of various overdispersed populations: centipedes, beetles, yeast, isopods and fleas; and he obtained very much closer fits than are given on the assumption of simple random distribution. His analysis disclosed, for instance, that only about 87% of the centipedes he sampled were present as individuals, and about 6.3% were present in pairs. Cole's method may not be easily applicable to plant populations where aggregation is due often to vegetative propagation, which gives many individuals in each group, and where the data are more likely to fit binomial than Poisson distributions. But as a first attempt to separate contagious distributions of organisms into components, Cole's work is important for plant ecologists, and it deserves serious attention.

An entirely different approach to the quantitative description of the distribution of species in a community has been made by Ashby and Pidgeon (2). By plotting lines representing equal frequencies of a particular species in a community, called "isonomes", these authors have shown that the distribution of a species may be resolved into centres of high frequency surrounded by a pattern of contours of lower frequency (Fig. 1). The technique has proved useful in reconstructing the history of colonisation of species in sandstone scrub in eastern Australia.

A few other recent contributions to the technique of statistical ecology are summarized in the following paragraphs.

Fisher (11) recently published a theoretical paper on the relation between the number of species and the number of individuals obtained in samples of organisms. The analysis has been applied successfully to the number of species in the samples of insects caught in a light-trap at Rothamsted (11), and it could be applied to the analysis of species-area curves in plant sociology.

Ramensky (21) published in 1938 an important monograph on

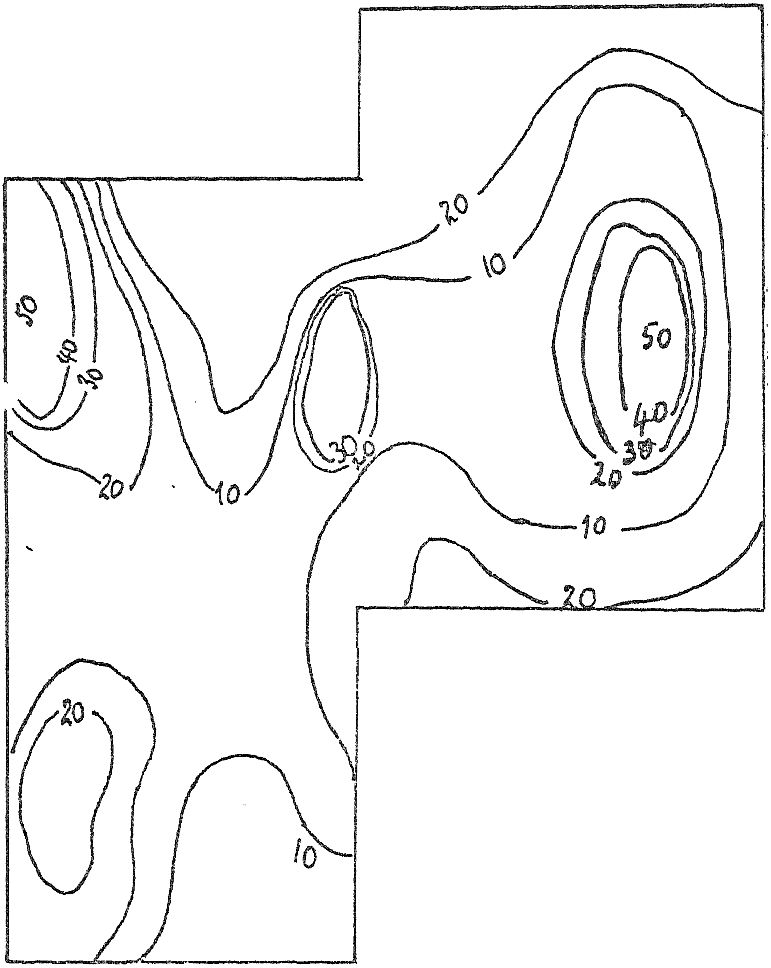

FIG. 1. Distribution of *Baeckea diosmifolia.* The numbers on the isonomes refer to percentage frequency.

ecological methods with special reference to pastures. He has devised an ingenious method of estimating percentage cover in closely cropped pastures: it consists of laying a lattice at random on the pasture, and comparing the pattern of vegetation under the lattice with a series of photographed patterns representing 20, 40, 60, *etc.*, per cent cover. The productivity of each component of the pasture is estimated by multiplying the percentage cover of each species by a factor which is derived from the regression of cover on total bulk of the species.

Pechanec and Stewart (17) have demonstrated a simple way of improving the efficiency of random sampling in pasture research. Instead of sampling at random over the whole area to be investigated, these authors divide the area into a certain number of blocks, and sample at random within each block. They are then able to separate out in their analysis the variance due to blocks and the variance due to error within blocks. The benefit of this simple procedure is evident from data published in their paper.

Vestal (26) has attempted to improve the precision of subjective estimates of density and cover, such as the familiar scales used by Hult, Sernander, Norrlin, Braun-Blanquet, and others. The "classes" of these scales are, of course, distorted measures of density. To minimise this distortion, Vestal proposes to substitute classes arranged in arithmetical or geometrical progressions. Such a proposal would indeed minimise one of the errors of these subjective methods, but it would still leave the methods open to the other objections which have been raised against them.

Penfound (18) has studied the way in which frequency is affected by quadrat size, cover and number of species. He used coloured cards, but his methods could be extended to field studies. He concludes that cover is the most reliable measure of plant distribution, since estimates of cover do not depend on the size of the quadrat used for sampling; but this conclusion does not meet the objection that estimates of cover are inevitably in some degree subjective. This objection can be met in part, at any rate, by working out a regression between percentage cover and some suitable measure of cover, *e.g.*, crown weight of leaves, as Kittredge (13) has done.

Among miscellaneous contributions to the technique of statistical ecology may be mentioned the work of Bauer (3) who reached, with the aid of 1,000 coloured discs, the not unexpected conclusion that transects can be used instead of quadrats for sampling communities; the work of Billings (4) who calculated an interesting regression between the organic matter of the soil and the age of trees in a *Pinus echinata* forest, the work of Dice (10) who measured the degree of "ecologic association" between species by a technique similar to that described by the present writer (1); and the work of Lynch and Schumacher (14) who used the method of probits to sample regenerated forest.

Conclusions about plant distribution. It is evident from the foregoing paragraphs that the technique for studying plant distribution has improved in the last 11 years. What advances in ecology have followed from this improved technique?

The most noteworthy advance is the knowledge that individuals are not, as a rule, normally dispersed, and that over-dispersion is much more common than under-dispersion. In large areas over-dispersion is to be expected, owing to heterogeneity of soil and micro-climate, which will have the effect of localising individuals in the more favourable habitats; but Blackman (5) has shown that over-dispersion occurs on grassland plots as small as 18 by 15 feet, where one might expect uniformity; Singh and Chalam (22) and Singh and Das (23) have shown that many weeds on a fallow wheat field are over-dispersed; and Pidgeon and Ashby (19) have shown that in a desert community, under uniform conditions of climate and soil, 68% of the species show non-random distribution. It seems to be established that although the occurrence of any particular species in a habitat may be due to chance, as Palmgren (16) has ably shown, and although, as Clapham (8) has suggested, groups of plants may be randomly distributed, yet individual plants or shoots are usually over-dispersed.

This habitual over-dispersion is due to one or another of several causes, some still obscure. Obviously plants which spread by rhizomes or runners or heavy seeds will be over-dispersed, although the amount of over-dipersion for some rhizomatous plants is surprisingly small. However, over-dispersion is present in species where there is no obvious biological reason for it, and indeed both Blackman and Pidgeon and Ashby, found that the same species may be randomly distributed in one habitat and over-dispersed in another. Thus *Campanula barbata* grew randomly in three plots and non-randomly in 10 plots; and *Senecio campestris* grew randomly in 12 plots on chalk downland and non-randomly in three plots. This evidence points to a conclusion, which would be important if it were confirmed, that the mode of dispersion of a particular species is not entirely a property of the species, but is dependent also upon the habitat.

There is some evidence that one habitat factor, namely, density of plants, affects dispersion. Thus Clapham (8) records the following values for *Senecio Jacobea:*

	Mean density	Relative variance (value for random dispersal = 1)
Sparse	1.67	3.04
Intermediate	3.80	7.95
Dense	12.44	6.09

Pidgeon and Ashby (19) record a relation between dispersion and density of Australian desert plants as follows:

Density per 100 metre²	Percentage of species	Percentage distributed: Randomly	
		Randomly	Non-randomly
0 - 50	57.7	20.0	37.7
51 - 100	11.7	3.5	8.2
Over 100	30.6	1.2	29.4

and they find that, for instance, *Eragrostis Dielsii* is randomly distributed at low densities and over-dispersed at high densities.

Singh and Chalam (22) report that weeds on arable land are, on the whole, randomly distributed when their density is low and over-dispersed when their density is high.

What little evidence there is indicates that a species which has no biological predisposition toward over-dispersion is randomly distributed when it first occupies an area. As its density increases it loses its randomness and becomes over-dispersed; and if it disappears from the community, owing to competition or the course of succession, its distribution may become random again during its relict stages. This evidence requires a great deal of confirmation, but if it is confirmed it will be an interesting problem to discover the cause of this cycle of dispersion which may accompany the colonisation, establishment and displacement of a species. One plausible speculation is that the over-dispersion of a species at high densities, when not caused by soil heterogeneity or by some obvious biological predisposition of the species, is caused by the presence at high densities of other species which are predisposed to aggregation. The fact that some species are over-dispersed may produce a secondary over-dispersion among the other species.

Some light is thrown on the interrelations of species in a community by the studies of Ashby and Pidgeon (2). From isonomes, whose construction is described along with Fig. 1, these authors have shown that the individual plants in sandstone scrub vegetation are not randomly distributed but are arranged in patterns of decreasing frequency around centres of high frequency. If the ground is completely covered there is an interdigitation between the patterns of the various species. Applied to some of the stages of succession on sand dune vegetation, the technique reveals that some species show a continuity of isonomes from one seral stage to another, while other species remain only in outliers as the succession proceeds. It would be a matter of some importance to know whether the centres of distribution disclosed by this analysis are themselves randomly distributed. Work on these lines is proceeding. What is needed for the next step is some modification of Cole's method of analysis suitable for studying the peculiar type of contagious distribution which is so widespread in plant communities.

ADDENDUM

Through the courtesy of Dr. C. B. Williams I have been able to read four papers of his in typescript: (i) The logarithmic series and its application to biological problems, Journal of Ecology, (ii) The logarithmic series and the frequency of occurrence of plant species in quadrats, (iii) The generic relations of species in small ecological communities, Journal of Animal Ecology, (iv) The logarithmic series and the comparison of island floras, Journal of the Linnean Society of London. All these papers will shortly appear in scientific journals. Some of the work embodied in these papers concerns the relation between the number of species and the number of genera in plant and animal populations; this topic is beyond the scope of the present review. However, the second of the four unpublished papers contains an important contribution to statistical ecology, namely an analysis of species-area curves by the logarithmic series $n_1, \frac{n_1 x}{2}, \frac{n_1 x^2}{3} \ldots$ where $n_1 =$ the number of species in the sample represented by only one individual, and where x is a constant, depending on quadrat size. Williams is able to give formulae for the theoretical relation between the area sampled and the number of species found in 1, 2, 3, . . . samples of the area. Using some of

Jaccard's data, Williams obtains linear relationships between the number of species and the logarithm of the number of quadrats; and he measures the richness of the flora by an "index of diversity".

Williams demonstrates neatly that Jaccard's method of comparing two communities by a "coefficient of community" (discussed in the present writer's previous review, 1936), is unsatisfactory, for the level of the coefficient is determined by two quite different causes: on the one hand, the richness of the flora (which is relevant), and on the other hand, the size of the area sampled (which is not relevant).

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RECENT DEVELOPMENTS IN SEED TREATMENT

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INTRODUCTION

Seed treatment occupies a prominent place among the agricultural practices that are continually subject to change and improvement. In a paper on seed treatment published in this journal in 1936 (100), the writer reviewed briefly the history of cereal seed treatment, the early use of copper sulfate and formaldehyde, the introduction of organic mercurials in liquid form, and then, with the discovery of copper carbonate dust as a bunticide, the gradual change to dust fungicides. The advantages and disadvantages of dust and liquid fungicides were enumerated, along with a discussion of the problems encountered in developing and testing new fungicidal materials. The cereal diseases amenable to control by seed treatment were listed as well as the fungicides being used at that time to combat them. Treatments for the seeds of other crops were also briefly mentioned. In conclusion, it was predicted that many changes in seed treatment, in both materials and methods, would result from the intensive research being carried on in that field. The fact that many such changes have been made in the past decade makes a review of the subject seem desirable and timely. A complete review of everything published on cereal or other seed treatments during that period will not be attempted; but an effort will be made to summarize much of the experimental data obtained and the general recommendations made, and to cite representative publications, many of which contain rather extensive bibliographies on the subjects discussed.

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Greater interest in, and acceptance of seed treatment as a farm practice in the past few years, are due to a number of factors. One of these is the tremendous increase in the use of hybrid seed corn. In 1936 only 3.1% of the total corn acreage in the United States was planted to hybrid seed. In 1946 this had increased to 67.5%. Since hybrid seed corn is almost invariably treated before planting, this change did much to popularize seed treatment with both seedsmen and growers.

With the advent of the late war and the urgent need for greater food production, seed treatment was advocated to increase yields by eliminating losses due to plant diseases. Extensive cooperative experiments were carried out by State and Federal agencies to test the different fungicides on the market and thus supply extension workers with reliable information regarding the materials and methods that were best suited for each crop. Inferior materials were weeded out, and many gradually disappeared from dealers' shelves. Improved devices for applying fungicides to seed were designed (*72*, *78*, *129*) for farm and commercial use. Among these are various home-made "batch mixers" and several types of continuous gravity treaters designed especially for applying volatile dust fungicides to seed. Several efficient commercial power treaters also have been designed and marketed, such as the Gustafson, the

Calkins and the Keck-Gonnerman. A recently designed seed treater applies the fungicidal dust to seed in the form of a slurry, a heavy suspension of a wettable form of the dust in water (126). This method of treatment adds from 0.5 to 1% of moisture to the seed, but this is largely on the surface and much of it soon evaporates. The slurry method eliminates the danger and discomfort attending the application of certain fungicides in dust form. It is now used to a considerable extent for treating seed of hybrid corn. These and other advances in materials and methods have created greater interest and confidence in the practice of seed treatment.

FUNGICIDAL MATERIALS

The trend toward organic fungicides which started over 30 years ago with the organic mercurials has recently extended into the field of non-mercurials. Prominent among these are the organic sulfurs and the quinones. Examples of the former are tetramethyl thiuram disulfide (Arasan and Thiosan or Tersan in the United States and Nomersan or Sulsol in England), ferric dimethyl dithiocarbamate (Fermate) and zinc dimethyl dithiocarbamate (Zerlate, Zimate and Methasan). Two quinone compounds that have shown great promise as seed disinfectants and protectants are tetrachloro-p-benzoquinone (Spergon) and 2-3 dichloro 1-4 naphthoquinone (Phygon). The early development of these and other promising organic compounds are discussed by Horsfall (70).

The fungicidal materials available at present for treating seed may be grouped into three general classes:

1. Mercurials, inorganic and organic.
2. Copper and zinc inorganic materials.
3. Non-mercurial organic compounds, metallic and non-metallic.

There can be added a fourth or miscellaneous group which includes sulfur, hot water, vapor, dry heat, gases, ultraviolet and infrared rays, and short wave or high frequency oscillations.

The principal fungicides falling into each of the above classes are listed below along with their chemical composition, their principal uses, their rates of application, and, if available and desirable, their source.

Mercurials

Inorganic Mercurials. These are not generally used for treating cereal seeds but are used for seeds of certain vegetables (62).

Mercuric chloride is used as a 1 to 1000 solution (four ounces in 30 gallons of water) for seed of crucifers, celery, cucumber and certain other vegetables including potatoes (62). Mercurous chloride or calomel is applied as a fine dust to seed of crucifers and onion (62).

Mercuric oxide (yellow oxide of mercury) is applied to seed potatoes as a dip treatment.

Organic Mercurials. These have been more effective in cereal disease control than any other class of fungicides.

Ceresan (2% ethyl mercury chloride), produced by the Semesan Division of the E. I. DuPont de Nemours and Company, is recommended for treating seed of cotton, peas and sugar beets. If other materials are not available it may be used also for wheat, oats, barley, flax and hemp. The usual rate is 2 ounces per bushel. It is marketed in South America as Granosan.

New Improved Ceresan (5% ethyl mercury phosphate), also a DuPont product, is used for small grains, sorghum, flax, hemp, cotton, peas and millet at a half ounce per bushel. It is applied also to tomato seed and to seed of a number of other crops. It is marketed in South America as New Improved Granosan.

Semesan Jr. (1% ethyl mercury phosphate), another DuPont product, is generally restricted to use on seed corn at the rate of one and a half ounces per bushel.

Barbak C (8% phenyl mercury cyanamid plus 1% cadmium oxide) is produced by American Cyanamid and Chemical Corporation and is applied to seed corn at one and a half ounces per bushel.

Corona Merko, an ammoniated hydroxymercury compound containing 3.5% metallic mercury, is made by Pittsburgh Plate Glass Co. for treating seed corn at two ounces per bushel. Its manufacture was discontinued during the war period.

Semesan (30% hydroxymercurichlorophenol), another DuPont product, is used for vegetable and flower seeds in either dust or liquid form. Its high mercury content renders it too expensive for treating cereal seeds.

Semesan Bel (12% hydroxymercurinitrophenol and 2% hydroxymercurichlorophenol), also DuPont-made, is used as a dip treatment for potatoes and sweetpotatoes.

Sanoseed Potato Dip (7.9% ethanol mercuric chloride) is made by Ansbacher-Siegle Corporation as a quick dip for seed potatoes.

Corona P. D. 7, a compound containing a combination of 5% mercury, 2% bromine and 7% phenol, is made by the Pittsburgh Plate Glass Company as a quick dip for seed potatoes.

Ceresan M, known in its experimental stage as DuPont 1452 (7.7% ethyl mercury p-toluene sulfonanilide), is marked by DuPont for treating seed of wheat, oats, barley, rye, sorghum, flax, hemp, cotton, peas, millet, soybeans and other crops for which New Improved Ceresan has been hitherto recommended. It is to be applied at the rate of a half ounce per bushel. Many experiment stations have found it to be about equal to New Improved Ceresan in effectiveness, but somewhat less vesicant and less toxic.

Hill's Merc-O-Dust (1.5% mercury as "mercurized hydroaromatic compounds" plus 2% formaldehyde) is manufactured by Seed-Treat Laboratories (Mobile, Alabama) and is recommended by them for seed treatment of cotton, small grains, flax and peanuts. The writer found it wholly ineffective for the control of seed-borne diseases in oats and sorghum (106, 107), and it has been found somewhat erratic as a treatment for cotton and peanut seed.

Parsons Seed-Saver Dust (5.15% para tertiary octyl phenoxy ethoxy ethyl dimethyl mercuric benzyl ammonium chloride, according to the 1946 label), is produced by Parsons Chemical Works for seed treatment of wheat, oats and barley at a half ounce per bushel. Its composition has varied considerably during the period 1940–1945 when its essential ingredients were mostly 10% or less of paraformaldehyde and some form of copper compound along with .01% of some mercury salt. No data on the relative effectiveness of the present product as a seed disinfectant are yet available.

Leytosan (7.2% phenyl mercury urea) is made by F. W. Berk & Co., London, England, and is sold in Canada but not in the United States. It is recommended for use on seed of cereals, flax and various other crops at a half ounce per bushel. In general it has been found less effective than N. I. Ceresan.

Mersolite (5% phenyl mercury acetate), also made by F. W. Berk & Co. (U. S. Subsidiary), has been used on cotton seed with inferior results.

Puratized N-5-E (10% phenyl mercuric triethanol ammonium lactate), sold by the Niagara Sprayer Company, controlled black scale in lily bulbs (93) at concentrations of from 1 to 500 to 1–4,000. It also has been found effective as a dip for Irish potatoes and sweetpotato. Puraturf is a form of this same compound produced especially for treating turf.

General Chemicals 668 (5% mercury trichloro ethylene), made by General Chemicals Company, has been tried as a seed treatment for cotton, and in general proved inferior to other organic materials already in use.

Copper and Zinc Inorganic Compounds

These compounds have undergone few changes within the past decade. Efforts have been made to develop effective organic copper fungicides but without much success (70). The copper-containing fungicides for seed treatment are, therefore, practically the same as those previously mentioned (100).

Copper carbonate, both the 50% and 20% brands, are still recommended for controlling bunt in wheat and the kernel smuts in sorghum by treating the seed at two and three ounces per bushel, respectively.

Basic copper sulfate, sold under various trade names by different manufacturers, continues to be used for the same purposes as 50% copper carbonate and at the same rate of two ounces per bushel. It contains about 50% copper and is made expressly for dusting seed or foliage.

Cuprocide or cuprous oxide (yellow or red) serves largely as a seed protectant to prevent seed decay and pre-emergence damping-off in vegetables. It is injurious to seeds of lettuce, crucifers and onions. It is not recommended for wheat and sorghum because carbonate and basic sulfate are more effective. Cuprous oxide varies in color, according to particle size, from yellow, which is the finest, through bright red to chocolate brown. The finer material is more effective because the coverage is better. Unless cuprous oxide is kept in an air-tight container or is given special treatment to prevent oxidation, it gradually changes to black cupric oxide which is relatively ineffective as a seed fungicide.

Copper sulfate solution (blue-stone or blue vitriol) is no longer recommended as a treatment for cereal seeds, although some farmers still use it as a bunticide. It is used also to some extent for treating seeds of beets, spinach, tomatoes and peppers (62).

The tendency of copper dusts to impede the flow of treated seed through the drill and to injure seed coats can be overcome by mixing it with about 25 per cent by weight of finely powdered graphite (8).

Zinc oxide (zinc white) is used on seeds of certain vegetables, such as crucifers and spinach, that are sensitive to cuprous oxide.

Vasco 4, a mixture of zinc oxide and zinc hydroxide, prepared by the Virginia Smelting Company, has been found effective on spinach and also on crucifers.

Non-mercurial Organic Fungicides

As previously mentioned, the organic sulfurs and quinones figure prominently in this class of compounds along with miscellaneous combinations of phenol, quinoline, chlorine, bromine and other materials. Some of these newer fungicides were first tested as sprays or dusts for foliage diseases but were later found promising also for seed treatment purposes. Undoubtedly other organic compounds will be added to the list of effective fungicides in the near future.

Arasan (50% tetramethylthiuram disulfide), a DuPont product, has been found effective for treating seed of wheat, corn, sorghum, rice, flax, peas, sugar beets, peanuts and many vegetables. Its outstanding feature is its beneficial effect on emergence from most seeds, due to its action as a seed protectant. It is not recommended for smut control in oats and barley. Tersan (formerly Thiosan) is a wettable form of Arasan used for spraying turf.

Fermate (70% ferric dimethyl dithio carbamate), also a DuPont product and used largely for foliage diseases, may be used also for potatoes, sweetpotatoes, peas, onions, cabbage, lettuce and some other vegetables. Its black color, however, is objectionable when treated seed is to be hand-planted in dark soil.

Zerlate (70% zinc dimethyl dithio carbamate), another DuPont product, is used mostly for foliage diseases and for turf.

Dithane (disodium ethylene bisdithiocarbamate), produced by Rohm and Haas Co., in addition to controlling certain foliage diseases has been used with some success for treating sweetpotatoes.

Spergon (98% tetrachloro parabenzoquinone) is made by the Naugatuck Chemical Division of the U. S. Rubber Company and was first used with excellent results as a seed protectant and disinfectant for peas and beans (31). It was later found effective also for seeds of a number of other crops, including corn, sorghum, cotton, flax, peanuts and many vegetables. It is not recommended for small grains. It controls bunt in wheat but is not entirely effective in controlling the smuts in oats and covered smut and stripe disease in barley.

Phygon, formerly U. S. 604 (98%, 2, 3, dichloro 1, 4 naphthoquinone), also a product of U. S. Rubber Co., may be used both as a spray for foliage diseases and as an effective dust fungicide for seeds of sugar beets, corn, sorghum and peanuts. It controls bunt in wheat but does not effectively eliminate some of the diseases in oats and barley that are controlled by the better organic mercurials.

Dow 9B, 50% zinc trichlorophenate, a product of the Dow Chemical Co., is recommended by the manufacturer for use on seed of cotton, corn, small grains, sugar beets, peanuts and vegetables.

Formaldehyde, the original non-metallic organic fungicide, continues in use to a gradually decreasing extent for seed treatment of cereals (especially oats), potatoes, beets, rhubarb and celery. It is useful also for sterilizing soil in hot beds, cold frames, flats and pots, for disinfecting bins and other containers where produce is to be stored and as a drip soil treatment for onion smut. It is cheap and easily prepared and doubtless will continue to be in demand. Its two shortcomings are its tendency to cause seed injury and its failure to act as a seed protectant.

Formaldehyde dust is no longer used to any appreciable extent as a seed treatment, because of its rapid deterioration and the consequent uncertainty of the results that can be expected from its use. The same applies to various paraformaldehyde products that were once on the market for seed treatment.

Other Seed Treatments

The hot water treatment remains the standard seed treatment for the flower-infecting loose smuts of wheat and barley. It is used also, to some extent, for treating seed of crucifers, onion, tomato and some other vegetables. This treatment is a disinfecting process only and does not serve as a seed protectant. Therefore it is desirable and often necessary to follow it with a chemical seed protectant to combat harmful fungi in the soil.

The use of gas for disinfecting large quantities of seed has often been suggested as the ideal method of seed treatment, but its feasibility has not been thoroughly demonstrated. One seed dealer in 1937 was found using chlorine gas to custom-treat local farmers' seed grain (110). Tests showed that the seed was being exposed to a concentration of less than 0.5% chlorine gas for about three minutes. Samples of this treated seed, planted in test plots, proved this treatment to be entirely ineffective. Laboratory and field tests showed that exposure to a concentration of 10% chlorine gas for one to two hours was required to control effectively bunt in wheat and covered kernel smut of sorghum. Smuts of oats and barley were not controlled by any concentration of gas or period of exposure that was not injurious to the seed.

The use of moist heat, supplied by a vapor heat machine, was described in 1944 (123) as being applicable to tons of seed exposed on a moving belt. Several advantages of this method over the hot water method were mentioned; critical temperature control is not essential, and tons of seed are easily treated and dried. Like the hot water treatment, however, this treatment would offer little protection against soil fungi, and a supplementary chemical seed protectant would in some cases be necessary.

Ultraviolet and infrared rays, short radio waves, Hertzian waves, diathermy, X-rays and other related devices have been tried at times as seed disinfectants, but none of these has reached the stage of practical application. Ultraviolet and infrared radiation were used on cotton seed with some success (151). Gier (47) used ultra short radio waves and ultraviolet light on several microorganisms and found it took 24 hours to kill the weakest fungus. Tverskoy (160) used short and ultra short radio waves to kill bacteria and fungi on agar or in salt solutions at 45° to 50° C., and suggests this means for killing the loose smut fungus in wheat kernels. English and Gerhardt (41) used ultraviolet (wave length 2537 Å from 30 watt "Uviarc" lamps) on spores of seven fungi associated with decay in sweet cherries. They concluded this offers small hope for practical control of such plant diseases.

CEREAL SEED TREATMENT

The principal reason for treating cereal seed is to obtain good stands of vigorous healthy plants that will produce increased yields of superior seed, free from systemic diseases, this to be accomplished without replanting or using an excess of seed. If the fungicide destroys harmful seed-borne pathogens and also protects the germinating seed from soil-inhabiting fungi, the above aim should be realized, provided the seed used is viable and of good quality. It should be emphasized that seed treatment is not a substitute for good seed. Increased stands, due to seed treatment, are not always reflected in increased yields, however, because the poorer stands from untreated seed are sometimes compensated for by increased tillering. But when weeds accompany poor stands and are suppressed by the more uniform stands from treated seed or when untreated seed produces weak diseased plants, the treatment usually results in increased yields. Seed treatment also may increase yields in wheat, barley, oats and sorghum by eliminating smut in a considerable percentage of the plants. One of the greatest benefits derived at times from the treatment of cereal seeds is the elimination of certain seed-borne fungi or bacteria that cause primary infection lesions from which the disease spreads to the leaves of other plants. Outstanding examples of such diseases are leaf blotch (*Helminthosporium avenae*) in oats, net blotch (*H. teres*) and spot blotch (*H. sativum*) in barley and probably bacterial stripe (*Pseudomonas andropogoni*) in sorghum. The spread by secondary infection in such diseases often causes a very heavy loss in the crop, even though only a very low percentage of the seeds sown were infected. Annual seed treatment of cereal seed is now generally recommended as a wise farm practice because the use of disease-free seed or of seed treated to eliminate certain diseases does not insure the production of disease-free seed for next year's crop. Air-borne spores causing various diseases, such as the loose smuts and *Helminthosporium* blights (15), may come from nearby fields and may contaminate the heads grown from treated seed. For this reason growers of certified seed have found it profitable to treat their seed every year in order to guard against this source of infection which may be sufficient to disqualify their fields for certification the next season.

Seed grain that is treated considerably in advance of seeding time, or excess treated seed that is carried over for the next year's seeding, is protected in storage to a great extent against weevils, flour beetles and several other grain-infesting insects, especially if the moisture content of the seed is low, 12% or less. While some dust fungicides may be actually toxic to these insects, many are sufficiently abrasive to the insect's abdominal covering to cause a fatal loss of its highly vital body moisture.

Seed laboratories are finding that seed treatment is a useful aid in arriving at a true evaluation of the germinability of some seeds. Various phases of this work are described by Porter (138).

Wheat. Bunt, it is believed, is the most widely observed seed-borne disease of wheat and is generally the principal disease for the control of which seed wheat is treated. Of all the seed-borne cereal smuts, bunt is probably the most easily controlled. New Improved Ceresan, Ceresan, Ceresan M, copper carbonate, basic copper sulfate, Arasan, Spergon, Phygon and a number of less commonly used fungicides, if properly applied, will control bunt satisfactorily where soil infestation does not occur (152). But some of these materials are inferior to the organic mercurials in eliminating organisms that are borne within the seed (56, 57, 113, 114, 124, 125), although some of them serve as excellent seed protectants. On the whole, organic mercurials, such as New Improved Ceresan, are recommended most frequently for wheat seed treatment.

Rye. Seed treatment of rye is not generally practiced in the United States, although it could occasionally prevent the spread of such seed-borne diseases as stalk smut and bunt. The treatments recommended for wheat are satisfactory also for rye.

Barley. New Improved Ceresan and Ceresan M applied at a half ounce per bushel are superior to other present chemical treatments for the control of covered smut, black loose smut, stripe disease and the seed-borne phases of net blotch, spot blotch and scab. Arasan, Spergon and Phygon have improved stands and reduced considerably the amount of covered smut in barley, but it is doubtful that these materials reach the more deeply seated infections in the seed as well as do the volatile mercury fungicides. Young and McClelland (173) report that N. I. Ceresan controlled barley covered smut and black loose smut caused by soil infestation from a previous crop, while formaldehyde and the standard hot water treatment failed to affect the amount of infection from this source.

Oats. The treatments recommended for oats are the same as those for barley—New Improved Ceresan and Ceresan M. Other materials may improve stands, but only the volatile mercury dusts and formaldehyde have been found consistently effective in controlling the oat smuts (59). *Helminthosporium* blight of oats, which caused serious losses in susceptible varieties in 1946 (125), also is combated in its seed-borne phase by the above organic mercury fungicides.

Contradictory results, reported at times by various workers with the same fungicides in oat smut control, may be due to differences in the methods of inoculation (59, 101, 103). Smut infection, induced by dusting smut-free seed with smut spores, is more easily controlled than is that brought about by natural inoculation in the field; while infection induced by the partial vacuum-spore-suspension method of seed inoculation is much more difficult to eliminate than is that caused by natural inoculation. Therefore naturally inoculated seed, it seems, is the logical material to use for testing fungicides in oat smut control (59, 101).

Corn. As previously mentioned, the great increase in the acreage planted to hybrid seed corn in the past decade did much to focus attention on corn seed treatment as a yearly practice. It has been demonstrated repeatedly that good sound seed corn, relatively disease-free, is not benefited by seed treatment unless cold wet weather follows planting. Since weather is an uncertain factor, seed treatment serves as "stand insurance". The treatments that have been recommended by various investigators are: Arasan, Semesan Jr., Spergon, Barbak C, Phygon, Dow 9B, Merko, Fermate and Ceresan M. Arasan and Semesan Jr. were found superior to Spergon and Barbak D for combating seed-borne *Diplodia zeae* (29, 68). Koehler (83) found Arasan and Spergon better than the mercurials on mechanically injured seed. On sweet corn Semesan Jr., Arasan and Spergon significantly increased emergence, plant vigor and height (35). In cooperative seed treatment experiments in eight southern States, 1943–1945 (116, 117, 118, 119, 148), stands were generally increased by treatment, although yields were not always increased, except from space-planted seeds. Certain treatments also reduced seedling infection due to *Fusarium moniliforme* and ear rot due to *Diplodia zeae.*

Sorghum. Like corn, sorghum benefits most from seed treatment when cold wet weather follows planting. At or below 20° C. seed of most sorghum varieties germinates very slowly (105, 108), while the food material in the seed is readily attacked and destroyed by seed-borne or soil-borne fungi, thus depriving the embryo of the wherewithal to produce a strong vigorous seedling. Treatment of the seed with an efficient chemical fungicide protects the seed from the action of these fungi until the seedling becomes established (18). In general, large soft seeds, like those of feterita and hegari, are most susceptible to seed decay because fungi are able to reach the starchy endosperm by direct invasion through the unbroken seed coats. In hard-seeded types fungus invasion is more or less confined to seeds with injuries in the seed coat caused mostly by threshing.

Sorghum seed treatment is desirable also for the prevention of the kernel smuts which frequently cause heavy losses. The fungicides generally recommended (17, 60, 102, 104, 107, 109) for treating seed of sorghum are: Arasan, Spergon, Phygon, copper carbonate, and basic copper sulfate, all applied at two ounces per bushel. To prevent smut in varieties having seed with persistent glumes it is necessary to use volatile dusts such as N. I. Ceresan or Ceresan M (107). These are applied at the rate of half ounce per bushel not more than six weeks before planting. Seed of some sorghum varieties, such as Leoti, is susceptible to "mercury poisoning" and should not be treated with mercury fungicides (105).

Sulfur, although fairly efficient as a preventive of kernel smuts, is not a seed protectant and at times has been reported as causing seed injury and reduction in emergence (17, 60, 107).

Rice. Seed treatment for rice is not generally recommended in the United States (25*b*, 159). Effects of seed treatments on emergence, seedling blight and yield have been too inconsistent to justify recommending their continued use. The hot water treatment (four hours at 54° C.) is recommended for seed heavily infected with *Helminthosporium oryzae* (143). N. I. Ceresan also is effective against this fungus and also combats injury due to several other fungi that may cause poor stands. The resulting increase in stands, however, is not necessarily reflected in increased yield (159). In 1945 and 1946, however, Cralley (25*a*) obtained increases in both stands and yields in several varieties as a result of seed treatment. Arasan and Phygon were particularly effective both years, while Dow 9B, Yellow Cuprocide and Spergon showed promise on the basis of one year's work.

Flax. Seed treatment of flax is more generally recommended and practiced at present than it was a decade ago. The negative results obtained in experiments with seed treatment at that time are attributed to the use of sound hand-threshed seed. It has since been observed that the greatest benefits are derived from seed treatment of machine-threshed seed that has been somewhat damaged in threshing (112, 145). In the British Isles seed treatment is practiced extensively for combating stem break and browning (*Polyspora lini*), seedling blight (*Colletotrichum linicolum*) and foot rot (*Phoma* sp.) (131, 132, 133). The materials used for this purpose are "organo-mercury powders" fixed to the seed by water or skim-milk. "Nomersan" (similar to Arasan) at 12 oz. per hundred lbs. almost eliminated seedling blight and partially controlled stem break and browning (131).

In the United States and Canada, N. I. Ceresan, applied at one to one and a half ounces per bushel, has been found to be the best treatment (54, 55). Ceresan M is almost equal to N. I. Ceresan but Arasan is not. Arasan, however, is superior to Spergon which has been found to cause abnormal leaf formation in flax seedlings (45).

Hemp. Although hemp, like flax, is not a cereal, it will be briefly discussed under that heading. Attention was directed to hemp seed treatment during the late war when efforts were being made to increase hemp production (63, 142). In greenhouse tests with relatively poor seed, increases in emergence up to 400% followed seed treatment with N. I. Ceresan at one to two ounces per bushel, while Arasan and Spergon were slightly less beneficial. Koehler (84) recommends N. I. Ceresan at one ounce or Arasan at two ounces per bushel as the best treatments. Wilsie and Reddy (169) obtained increases in stand with N. I. Ceresan, Spergon and Arasan, but these were rarely followed by increased yields.

SEED TREATMENT FOR OTHER FIELD CROPS

Forage Crops. Seed treatment for forage crops has received considerable attention in recent years, but the great number and diversity in genera and species in this group of field crops makes general recommendations difficult.

Henry *et al.* (64) controlled smut in slender wheat grass with N. I. Ceresan and Leytosan at a half ounce per bushel. Chilton and Garber (20) treated seed of 18 species of forage legumes—*Lespedeza, Lotus, Medicago, Melilotus* and *Trifolium*—and found N. I. Ceresan the best treatment. Highly significant increases in stand were obtained in some species and no differences or some injury in other species. Kreitlow (85) reported increased stands in Sudan grass with Spergon and in alfalfa with yellow Cuprocide. Allison and Torrie (5) treated seed of alfalfa and five types of clover with N. I. Ceresan, Spergon and Arasan at the rate of 1 per cent of weight of seed, but obtained no beneficial results. Wang (163) found N. I. Ceresan most effective for controlling smut in millet. Fischer (43) controlled head smut and stripe smut in Canada wild rye with N. I. Ceresan and Arasan, the latter at four ounces per bushel. Overdoses of N. I. Ceresan injured seeds and seedlings.

Experiments with soybeans, in general, show few significant or consistent benefits from seed treatment. Allington *et al.* (4) reported that in uniform tests at points in 18 States, seed treatment with N. I. Ceresan, Arasan and Spergon caused significant increases in stands but not in yields. Differences between treatments were not significant. Porter (137) and Koehler (82) obtained similar results. Sherwin *et al.* (150) reported increased emergence with N. I. Ceresan, Arasan and Spergon at 15°, 20° and 25° C. but not at 30° C. Heuberger and Manns (65) accelerated emergence and increased stand and yield with these same materials. Hildebrand and Koch (66) obtained similar increases in stand and yield of soybeans with Spergon, applied to poor quality or weathered seed, or seed of good quality but injured in threshing. In this case stand increases due to treatment were correlated with statistically significant increases in yield. Tervet (155) found that Arasan increased stand and vigor of soybean seedlings from seed treated before storage.

Cotton. Treatment of cottonseed, both mechanical and chemical, has increased tremendously in popularity since 1936. In North Carolina, for example, the area planted to treated seed increased from 24,000 acres in 1936 to over 600,000 acres in 1940 (61). In South Carolina an increase from about 4,000 acres in 1935 to over a half million acres in 1940 is reported. Similar reports of increased acreages planted to treated cottonseed have been received from other States.

Ceresan (2%) was at first the fungicide most widely used for cottonseed, but in recent years it has been largely replaced by N. I. Ceresan. Delinting with sulfuric acid has been replaced to a great extent by mechanical delinting or "reginning". The removal of the linters of seed fuzz from the seed generally increases both the rate and per cent of emergence (111). Delinted seeds can be planted more evenly than fuzzy seeds (58), and they germinate more readily. While delinting removes some of the seed-borne fungi, delinted seed, nevertheless, should be treated. Although seed treatment, especially with organic mercurials (98), eliminates injury due to seed-borne fungi and bacteria, it provides little protection against soil-borne forms of *Fusarium* and *Rhizoctonia* (96, 139, 166).

In recent years fungicides other than 2% Ceresan and N. I. Ceresan have been found effective for treating cottonseed. Lehman (97) in 1942 reported that treatment with Spergon increased stand and yield over untreated seeds and in 1945 (99) obtained better stands with Dow 9B than with N. I. Ceresan. In cooperative experiments in nine States in 1946 (7) Dow 9B and Ceresan M were equally effective, and generally superior to Fermate, Zerlate, Spergon and General Chemicals 668.

Sugar Beets. Damping off, both preemergence and post-emergence, continues to be the principal obstacle in obtaining satisfactory stands in sugar beet fields (100) ; hence the control of this disease complex is still the chief objective of seed treatment. Some of the materials that have been used for this purpose in recent years are: Ceresan, N. I. Ceresan, copper carbonate, Cuprocide, mercuric iodide plus a mixture of copper carbonate and Celite 1 : 14, mercuric chloride plus copper carbonate and urea (equal parts), Ceresan plus copper carbonate 2: 1, N. I. Ceresan plus copper carbonate 1 : 1 (23, 24, 46, 88, 94, 140). Leach (88) and Kotila (unpublished data) also found Phygon among the more promising fungicides in recent tests, while Spergon gave inferior results in several experiments. In California cuprous oxide was found more effective against *Pythium* than were the two Ceresans which, in contrast, were more effective than cuprous oxide against *Rhizoctonia* and *Phoma* (90). N. I. Ceresan and Arasan controlled seed-borne bacterial blight (*Phytomonas aptata*) in California and Oregon (6).

During the war emergency the widespread use of sheared, or segmented, sugar beet seed as a labor-saving measure brought about a decided change of attitude with regard to sugar beet seed treatment. Whereas a considerable loss of seedlings from damping-off may be tolerated when whole seed is planted at a heavy rate, these losses assume very great importance with the relatively sparse seeding rates employed when sheared seed is planted. The advantages resulting from the use of sheared seed can be obtained only if the individual seed pieces are planted about one or two inches apart in the row, so that the resulting stand can be thinned mechanically or at least with a minimum of hand work. Therefore, to obtain even stands, seed treatment has been generally recommended since the introduction of sheared seed (23, 46, 89).

The pelleting of sheared or segmented seed by use of pill-making techniques has been advocated by some in order to produce uniformly sized seed pellets that will facilitate uniform planting rates. Fungicidal and nutrient materials are used in coating the seed, so that disease control may be combined with vigorous early growth (23). In areas where soil moisture is an uncertain factor after planting, this coating may interfere with water absorption by the seed and hence may delay germination. In irrigated areas and in regions of abundant rainfall this does not occur.

The extent to which seed treatment controls damping-off or "black root" in sugar beets depends to a considerable extent on the type of fungus population of the soil. This in turn, it has been shown, is governed largely by proper soil management, especially the kind of crops previously grown. The disease is more severe when beets follow beets, clover or alfalfa in the rotation, and least when beets follow corn (24, 94). Effective soil drainage and the use of soil amendments (24, 1), such as phosphate fertilizers and lime, also help to reduce damping-off injury. Seed treatment often may prove effective against early attacks of the fungi causing damping-off, but, if growth is retarded by excessive soil moisture, low fertility or other forms of poor soil management, the good initial stand will not be maintained. With well drained, adequately fertilized soil, and with a proper crop rotation, very definite improvement of sugar beet stands may be expected from seed treatment with the better fungicides.

Vegetables. The problems encountered in attempts to control vegetable diseases were mentioned briefly in the previous review (100), and few, if any, of these have been completely eliminated. Damping-off probably continues to be the greatest difficulty to be combated in getting good stands, although in some crops certain seed-borne diseases are serious hazards. The nature and cause of damping-off in vegetables, and measures for combating it before the advent of non-mercurial organics, were ably discussed by Kadow and Anderson (79) and by Horsfall (69).

The chief chemical materials used for vegetable seed treatment ten years ago were cuprous oxide, zinc oxide and hydroxide, mercuric chloride, formaldehyde and certain organic mercurials such as Semesan and for potatoes Semesan-Bel. The most outstanding materials added to this list in recent years are Spergon, Arasan, Fermate, Phygon, yellow mercuric oxide, and yellow cuprocide, an improved cuprous oxide. Ceresan and N. I. Ceresan also are now used to some extent for vegetables.

Detailed and lengthy directions for treating seed of the various vegetables with the proper fungicides will not be attempted here, for such information is available in a number of State and Federal publications (19, 21, 62, 120, 146, 147, 158, 168). Frequently reports from different regions indicate that a given fungicide may yield excellent results on a certain crop in one area and not in another. This may be due to differences in climate, soil, variety, rate or manner of application, or other factors. However, Gould (49) reported that in his experiments in Washington "materials superior in one area were generally superior in all, despite differences in soil type, rainfall etc". He found (48) Arasan, Spergon, Ceresan and Semesan most consistently effective for seed of 15 vegetables, with Arasan most suitable for chenopodiaceous crops and Spergon for legumes. Taylor and Rupert (153) found Arasan the most generally effective fungicide, with Fermate next, but both injured lettuce seed for which cuprous oxide is recommended. Fermate, because of its black color, was found objectionable for planting seeds by hand in dark soil. In extensive cooperative tests of different fungicides on seed of various vegetables by the Seed Treatment Committee of the American Phytopathological Society in 1943 (146) and 1944 (147) Arasan proved more generally effective than the other fungicides tested, especially on beets, carrots, sweet corn, tomato, spinach and to some extent on cucumber and onion. Spergon was best for edible soybeans, lettuce, peas and lima beans. Zinc oxide frequently excelled for spinach. In experiments in Florida (158) in which seed of 21 kinds of vegetables were treated with six fungicides, the fungicides rated first and second, respectively, the following numbers of times: Arasan, 13 and 2; Spergon, 3 and 6; Semesan, 0 and 11; cuprous oxide, 2 and 1; zinc oxide, 3 and 0; Ceresan 0 and 1. Gould (49) found Phygon very effective on seed of beans, spinach, squash and sweet corn. Spergon and cuprous oxide were excellent for lettuce, while Arasan and Fermate caused injury (153) to seed of this crop. The control of onion smut by means of Arasan or Fermate instead of formaldehyde (38, 136) has met with some success, but in severely infested soil formaldehyde still seems to be the only logical fungicide.

The customary treatments for tomato seed in the past, solutions of copper sulfate, mercuric chloride or Semesan, have been largely replaced by treatment with N. I. Ceresan. At first it was applied as a five minute soak in a solution of ethyl mercury phosphate (1 to 32,000 (144) to 1 to 24,000 (161) made by mixing one gram of N. I. Ceresan in 1,600 to 1,200 cc of water). Later it was found that dusting the seed with N. I. Ceresan at the rate of 1 lb. to 200 lbs. of seed was equally effective (34). The greatest benefits from seed treatment are obtained when cool wet weather follows planting (130).

Seed treatment for peas is a practice generally recommended. The materials used include Spergon, Arasan, N. I. Ceresan, yellow cuprocide, Semesan, Phygon and Fermate (44, 92, 95, 121, 149). Spergon frequently has produced the best results from seed treatment of peas and beans. However, at times some of the other fungicides, especially Semesan (95), have proved superior, particularly against root rot caused by *Pythium ultimum* (92). Cuprocide has been observed at times to cause necrosis, delayed absorption and reduced seedling growth. No treatment controlled rot of cotyledons and epicotyls by *Rhizoctonia solani* (92).

Peanut seed treatment, in general, has resulted in greatly increased stands, especially from machine-shelled seed (162, 170). Arasan in a number of experiments seemed to be somewhat superior to two per cent Ceresan, Spergon and yellow cuprocide. Phygon was best in some experiments. In cooperative experiments in three States (42) in which Arasan, two per cent Ceresan, Phygon, Dow 9B, Spergon, Cuprocide, Ceresan M and General Chemical 668 were used, 2% Ceresan was reported best in South Carolina, and all were beneficial in Alabama and Virginia, especially on machine-shelled seed.

Potatoes, although not grown, as a practice, from true seed, may be included in the discussion of vegetable seed treatment, as the tubers used as seed are frequently subjected to treatment. Potato tubers may harbor a host of disease pathogens. Jensen and Livingston (77) list 20 diseases affecting the tubers, and Dykstra (40) mentions 15 or more potato maladies that may be transmitted by means of the tubers. Very few of these diseases, however, are amenable to control by seed treatment. Scab and Rhizoctonia, or black scurf, are the chief targets at which seed treatment is aimed, as these may be carried on the surface of the tuber. *Fusarium* seed-piece decay also responds to seed treatment (32). However, seed treatment does not prevent scab and *Rhizoctonia* when they are soil-borne, nor is it effective against diseases that are carried in the interior of the tuber.

Hot formaldehyde (1–120 at 125° F. for four minutes) is generally recommended for the control of scab (77), while mercury treatments are preferred for *Rhizoctonia.*

Acid mercury (six ounces of mercuric chloride, dissolved in one quart of commerical hydrochloric acid, added to 25 gallons of water), cold mercuric chloride (1 to 1,000 solution), hot mercuric chloride (126° F.), and yellow oxide of mercury (one pound of mercuric oxide in 30 gallons of water) are the four inorganic mercury treatments. To avoid injury to the tubers certain precautions are essential when using the formaldehyde and mercury treatments (62, 77).

Several organic mercury treatments also are available for the control of *Rhizoctonia* in potatoes. Among these Semesan Bel, Sanoseed and Corona P. D. 7 have been most frequently used. The advantages of these treatments are ease of preparation, shortness of treatment and absence of injury.

Materials for treating potatoes to prevent sprouting in storage may be regarded to a certain extent as seed treatments. The methyl ester of naphthaleneacetic acid has been found effective for this purpose (157). It is applied at the rate of 0.9 gram per bushel in dilute alcohol or lanolin emulsion sprays, in talc or walnut shell dusts or in paper strips. It may extend the period of marketability of tubers for several months. Its application to seed potatoes has not yet been recommended.

Sweetpotatoes are treated for protection against seed-borne black rot, scurf and stem rot, and to combat to some extent certain soil-borne pathogens, such as *Pythium* sp., *Rhizoctonia solani* and *Sclerotium rolfsii.* The most generally recommended treatments in the past have been an eight to ten minute soak in a 1–1,000 mercuric chloride solution or a dip in Semesan-Bel soultion (1 lb. to 7½ gallons of water). Both of these, while effective, sometimes delay or reduce sprout production. Therefore a search for effective but less injurious materials has led to recent experiments which included tests with Fermate, borax, Spergon, Phygon, Zerlate, Puratized N5–E and 177, Isothan Q–15 (lauryl isoquinolinium bromide) and others (33, 75, 76, 146). Nusbaum (146) reported encouraging results with Fermate and borax. Jeffers (75) found Fermate only slightly effective, while Puratized N5–E and 177 and Isothan Q–15 were in a class with mercuric chloride and Semesan-Bel in effectiveness, and borax, Zerlate, Spergon and Phygon were moderately effective. It seems that more experimental work is necessary before definite recommendations can be safely made regarding the use of some of these materials.

Some work has been done on the treatment of roots, rhizomes, bulbs and corms of certain ornamentals that are propagated by these underground parts. *Fusarium* rot of narcissus bulbs in storage may be reduced by treatment with 2% Ceresan and N. I. Ceresan (167). McClellan and Stuart (115) found Arasan, N. I. Ceresan and Spergon effective for controlling bud scale rot in Easter lilies. Le Beau (93) controlled black scale in bulbs of *Lillium longiflorum* with Puratized N–5–X and N–5–E. Dosdol (39) states that mercury treatments (calomel, Semesan, yellow mercuric oxide, mercuric chloride and acid mercuric chloride) are effective for *Botrytis* crown rot in iris rhizomes. Creager (26) found a 15 minute dip in N. I. Ceresan (half lb. in 25 gallons of water) and a three-hour soak in Lysol (one pint to 25 gallons of water) effective against *Fusarium* brown rot in gladiolus corms.

SEED TREATMENT WITH HORMONES

Whether or not beneficial results follow the application of plant hormones or growth-promoting substances to seeds is still a debated subject. An interesting review of the development of and early work on this phase of seed treatment was given by Mitchell (128) in 1940. He conducted extensive experiments to test the value of applying different growth substances to seeds and plants in various ways with a view to determining whether they would promote growth, increase yield or advance the time of flowering. He used different concentrations of indoleacetic, naphthaleneacetic, indolebutyric and indolepropionic acids, indoleacetamide and naphthaleneacetamide in various ways on seeds, roots, stems and leaves. The plants were grown in the field and in the greenhouse, and the plantings were sufficiently replicated to permit statistical analysis of results and to minimize individual variation in plants. The results failed to show that these substances had any effect on total plant growth, time of flowering or yield in these experiments. Ireland (74), in a brief review of this subject, suggests some reasons for the diversity of results obtained by different investigators. Among others he mentions variation in intensity of ultraviolet radiation and the presence or absence of apparently essential complementary substances.

Barton (11) tried seven growth-promoting materials on seeds of five cereals, nine kinds of vegetables, 11 kinds of flowers and four kinds of forage crops and obtained no favorable effect on germination or subsequent growth. She also failed to effect the dormancy of certain seeds with hormones (10). Crosier and Heit (27) dusted seeds of beans, corn and peas with three growth-promoting substances before placing them in the germinator and reported no effect on germination or on fungus growth. Crosier, Patrick and Weimer (28) obtained negative results on germination, winter survival and seedling weight in cereals. Hopkins (67) used 2.5 and 5 ppm. indoleacetic acid on seed of barley and obtained no effect on germination or yield of grain, but at suboptimal moisture the plant height was increased 3% and the dry weight of straw 10%. Hwang and Pearse (73) treated seed of oats and broad bean in dilute indoleacetic acid (2, 20 and 200 ppm) and obtained no increase in dry weight of seedlings. Higher concentrations retarded growth. Lafferty (87) reported no benefits to stand, yield or quality from soaking barley seed in dilute indoleacetic and naphthaleneacetic acids. Templeman and Marmoy (154) applied solutions of sodium-alpha naphthylacetate to lettuce, mustard and tomato seed, and alpha naphthylacetic and beta indoleacetic acids to seeds of wheat, oats barley and sugar beet but obtained no increased growth. Youden (172) treated wheat and soybean seed with four hormones without significant benefit of any kind. A number of other workers have reported negative results after using hormones on seeds of grasses and clover (2, 3, 36), corn, soybeans, oats, barley, sugar beets and cotton (11, 37, 80, 97, 135, 171).

Reports of benefits derived from applying plant hormones or growth-promoting substances to seeds are numerous. Bonner (13) reports that soaking pea seeds in a concentrated solution of Vitamin C caused the seedlings to increase in dry weight 35% faster than the controls. He also used Vitamin B_1 in other experiments (14). Thimann and Lane (156) state that treating seed of wheat or oats with a low per cent of auxin accelerates rate of root elongation, while a high per cent inhibits it. McRostie *et al.* (122) treated seed of winter wheat with indoleacetic and naphthylacetic acids at two to ten and two to 100 ppm., respectively, and obtained significant differences in straw production, yield, bushel weight and nitrogen content of grain. Grace (50) dusted seed of wheat and barley with a number of materials and reported stimulation of both root and top growth. Indoleacetic acid and indolebutyric acid (2 ppm) increased root length 65% and 55%, respectively, while a combination of these, at two ppm each, increased it 102%. Hurd-Karrer (71) used these and other materials on seed of Leapland wheat (2, 5, 10, 20 and 50 ppm) in an attempt to duplicate Grace's results but obtained only negative results.

Grace (51) later reported that sulphanilamide and a number of chemicals, other than the commonly known auxins, promoted growth. He found also (52, 53) that naphthylacetic and indoleacetic acids counteracted injury to wheat seed caused by formaldehyde treatment. Wark (164) reported similar results with several hormone materials in reducing injury to seed of wheat by ethyl mercury phosphate. Croxall and Ogilvie (30) treated seed of seven varieties of peas and two of beans with mercury treatments and cuprous oxide with and without the addition of growth hormones. They report that under certain conditions the addition of the growth hormones overcame the slight injury due to mercury treatment and also increased the dry weight.

The lack of agreement in the results reported by different workers on the use of these growth-promoting materials on seeds indicates that our present knowledge concerning their use in seed treatment needs to be greatly increased before definite recommendations can be made. Highly beneficial results of commercial importance are being obtained with them in inducing root formation on cuttings, preventing fruit drop in apple orchards and inducing fruit formation without pollination in tomatoes, squash and some other crops. This makes it reasonable to believe that the germination of seeds and the early development of seedlings likewise can be improved by their proper use. As one worker (128) aptly states, "It is to be regretted that numerous fantastic and unfounded claims have been made because of insufficient evidence, coupled with too great enthusiasm".

SYNERGISM AND ANTAGONISM

The subject of synergism and antagonism between different fungicides and insecticides applied to foliage has received considerable study and is ably reviewed by Horsfall (70). In the field of seed treatment, however, this topic has received much less attention. Mention has been made of reports that certain hormones reduced injury due to seed treatments (30, 52, 53, 97, 164). McClellan and Stuart (115) report that Arasan prevented inhibition of bulblet formation and root development in lily bulbs induced by treatment with N. I. Ceresan. A number of workers have applied mixtures of different fungicides to seed but usually without much evidence of synergism. For example, Dow 9B was not improved by addition of Spergon or Zerlate; nor Ceresan M by addition of Fermate (7). Spergon plus N. I. Ceresan on hemp seed was better than Spergon alone (169), due, probably, to the additional action of the latter which, unfortunately, was not used separately for comparison.

Following the publication of a number of glowing reports on the results of hormones applied to seeds, manufacturers of fungicides considered the advisability of adding growth-promoting substances to their products used for seed treatment. A study of the available data on the subject, however, failed to show sufficient agreement in the results to indicate definitely the kind or amount of hormone to be added. The possibility of antagonism between the hormone and the fungicide (70) had to be considered. The paucity of information on the subject made it seem advisable to postpone this step for the present.

In the fall of 1946, experiments[2] were started to study the effect of mixing each of two insecticides (magnesium oxide and DDT) with each of eight fungicides (N. I. Ceresan, Ceresan M, Arasan, Spergon, copper carbonate, Phygon, Semesan Jr. and Barbak). The materials were mixed in a ball mill in the proper proportions so that when the mixtures were applied to the seed each fungicide and each insecticide was applied at the generally recommended rate per bushel. The insecticidal value of the various dusts and mixtures was tested by applying them to wheat subsequently infested with adult rice weevils and confused flour beetles. The fungicidal effectiveness of the dusts was tested by applying them to seed of wheat, barley, oats, corn and sorghum and observing their effect on emergence and disease control.

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[2] In cooperation with R. T. Cotton, Entomologist, Bureau of Entomology and Plant Quarantine, and P. E. Hoppe, Pathologist, Bureau of Plant Industry, Soils and Agricultural Engineering.

N. I. Ceresan and Semesan Jr. reduced the insecticidal action of DDT somewhat whereas the other fungicides had no apparent effect on it. The insecticidal action of magnesium oxide was apparently unaffected by any of the fungicides.

The incorporation of pure DDT into the fungicides failed to affect their fungicidal value appreciably. Hoppe reported, however, that 3% DDT in mixtures with the two dusts, N. I. Ceresan or Ceresan M, which are known to be injurious to corn, reduced the injury to a greater extent than would be expected from such a light dilution. A well replicated experiment seemed to confirm this reaction between DDT and two mercurials.

Magnesium oxide greatly reduced the beneficial effects of copper carbonate, and to some extent of Spergon, on seedling emergence. It reduced the effectiveness of copper carbonate in the control of kernel smut in sorghum, allowing 20% infection compared with 1% for copper carbonate alone, 37% for magnesium oxide alone and 60% for no treatment. Horsfall (70) mentions a similar reaction between magnesium oxide and cuprous oxide. Hoppe reported that magnesium oxide reduced the efficiency of the dusts used on corn, but proved beneficial in off-setting injury by Ceresan M and N. I. Ceresan, especially when these mixtures were applied by the excess method. In a subsequent experiment magnesium oxide added to Dow 9B greatly reduced the effectiveness of that fungicide in the control of kernel smut in sorghum, but when added to sulfur, seemed to increase the fungicidal value of the latter material.

Pyrophyllite, containing 3% DDT, when added to Dow 9B reduced the control of sorghum kernel smut from 0.3% to 40%, with 60% in the controls from untreated seed. Whether this was due to the DDT or to the diluent was not determined.

These few cases of reaction between fungicides and hormones, insecticides, diluents and other fungicides suggest that caution must be used in mixing such materials in farm practice without preliminary experimentation.

A commercial fungicide for seed treatment usually is a well balanced combination of active ingredient and proper diluent with the possible addition of such materials as wetting and dispersing agents, dyes and other materials in the proper proportion. The addition of some other materials may result in chemical reactions and the formation of new compounds that are ineffective as fungicides or are injurious to the seed.

EFFECT OF STORAGE ON TREATED SEED

Freedom from injury after prolonged periods of storage, along with protection against weevils and rodents during storage, were among the advantages claimed for the copper carbonate treatment of wheat over 20 years ago, provided, of course, that the seed was of relatively low moisture content. This applies probably to most non-volatile fungicidal dusts. However, the possibility of injury to seed, stored for long periods after treatment with organic mercurials or other volatile dust fungicides, has been frequently mentioned. A general review of the literature on this phase of seed treatment indicates that such injury is dependent on several factors: (*a*) the moisture content to the seed; (*b*) the rate at which the fungicide is applied; (*c*) the length of the storage period; (*d*) the temperature, humidity and aeration during storage (141); (*e*) the kind of seed (seed of some genera, species or even varieties are more susceptible to chemical injury than are seeds of others); and (*f*) the condition of the seed coat (cracked or broken seed coats are conducive to seed injury).

Koehler (81) treated seed of wheat, oats and barley containing less than 13% moisture with N. I. Ceresan at rates of a half and a quarter ounce per bushel (also an eighth ounce for oats) and stored it for periods ranging from one to 67 days. In general, the seed treated at the half-ounce rate produced the better yield when sown one day after treatment, while the seed receiving the lighter application produced the better yield when sowing was delayed two weeks or longer after treatment. In all cases, however, the yield from treated seed was considerably greater than that from untreated seed.

He also treated different lots of seed corn with each of several mercury disinfectants at one and a half ounces per bushel and subjected the seed to different periods of storage, ranging from one day to one year. Contrary to results obtained with seed of small grains, treated seed corn stored for one year and that stored for one day when planted showed no significant difference in yield. He found that high moisture content—over14%—in stored corn, treated or not treated, is inadvisable, and suggests also that the seed be stored in a dry closed building, slightly heated during the cold months.

Baylis (12) treated samples of seed wheat with Agrosan G. and Ceresan U. T. 1875 A at dosages of two and four ounces per bushel and stored it for 12 months. The moisture content of the seed samples ranged from 12.8 to 16.7 per cent. In general, the lower the moisture content of the seed, the higher was the field germination of both treated and untreated seed. The treated seed in all moisture classes germinated better than the untreated seed.

Brett and Dillon Weston (16) dusted seed of wheat, oats and barley with organic mercury disinfectants and state that seed of good quality and proper moisture, dusted at the correct rate and stored properly, was not injured by one year's storage. Heavier dosages reduced germination as also did high temperature and a too high moisture content. Wheat was injured most and barley least.

Wark (165) in South Australia treated separate portions of barley seed and Rance wheat seed with six mercurials, one organic sulfur and two copper dusts and stored samples of each at 0°, 10°, 20° and 30° C. Germination tests were made after treating and after storage for two, four, six, and eight weeks. The mercury dusts reduced germination of wheat seed after storage, especially at the higher temperatures, but improved that of barley.

Colhoun (22) in Ireland dusted flax seed with Nomersan (Arasan) and observed no injury to germination after 18 months storage. The short wet treatment, however, resulted in reduced germination after storage.

Muskett and Colhoun (134) dusted flax seed containing less than 10% moisture with N. I. Ceresan at a dosage of 12 ounces per 100 pounds. No effect on germination was noted after eight weeks storage, but after 18 weeks the treated and untreated seed germinated 75 and 95%, respectively. Seed with a moisture content of 14%, similarly treated and stored, germinated 46% after 14 days, compared with 65% for untreated seed.

Millikan (127) treated flax seed with N. I. Ceresan at four ounces per 100 pounds of seed and stored it in an air-tight container for one month. The treated seed produced 52% normal seedlings as compared with 89% for untreated seed similarly stored.

Treated and untreated seed of five varieties of sorghum was stored at 20° C. and 70% relative humidity for 180 days (107). In germination tests in steamed compost soil after two, 20, 40, 80 and 180 days storage, Arasan, Spergon and copper carbonate either improved the average emergence or did not impair it, compared with the untreated control. N. I. Ceresan reduced the average emergence significantly in three varieties and improved it in one. In unsterilized soil the protective effect of N. I. Ceresan outweighed its injurious effects on certain varieties so that it caused increases in the average emergence in all cases, three of which were significant. The other three treatments were consistently and significantly beneficial to emergence.

Kreitlow and Garber (86) treated seed of alfalfa, clover and Sudan grass with N. I. Ceresan, Semesan, Arasan, Spergon and yellow cuprocide and stored it in open and in closed containers at 10° and 25° C. for 30 months. After one month the germination of Sudan grass treated with N. I. Ceresan was noticeably reduced, and after six months it was completely inhibited, while the other treatments caused no injury. Seed of clover and alfalfa showed no appreciable injury from any of the treatments. At 10° C. all seeds stored in closed containers germinated better than those stored in open containers.

Vaughan (161) states that tomato seeds treated with N. I. Ceresan may be stored safely from one season to the next.

Sugar beets (91) dusted with cuprous oxide or Ceresan and stored for 12 months showed no damage even at excessive dosages, but addition of 6% moisture to treated seed caused injury after 30 days storage.

N. I. Ceresan (84) applied at two ounces per bushel injured hemp seed after three months storage if the seed contained 10% or more of moisture. Arasan and Spergon caused no injury, and all three materials greatly improved germination under the right conditions of treatment and storage.

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SOMATIC MITOSIS IN THE ROOT TIP OF *ALLIUM CEPA*—A REVIEW AND A REORIENTATION

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INTRODUCTION

Despite the obvious truism that a proper understanding of somatic mitosis is basic to the whole field of biology, and particularly to cytology, embryology and genetics, such understanding is confined, for the most part, to the professional cytologist and cytogeneticist, and is not to be found in elementary texts, which are about a quarter of a century behind the frontiers of research. Furthermore, there are several important parts in the picture which have remained to the present time unfilled by the cytologists themselves.

Three forms are generally used to illustrate mitosis in elementary courses and in advanced histology and cytology—somatic cells of whitefish, gonial cells of grasshopper follicle, and meristematic cells of onion-root tips. Whitefish preparations are excellent for an over-all picture showing the asters and spindles, and the four classical stages, *viz.*, prophase, metaphase, telophase and interphase, but the chromosomes are too small for detailed study. The situation in the grasshopper will be treated in another series of papers. My present topic is onion-root tip mitosis, for which there is at present no complete and up-to-date account in the literature, despite the great importance of this form in both pedagogy and research. Chromosome morphology and karyokinesis have been described in the genus by various older workers (1, 2, 26, 27, 57–78), and latterday authors (16, 42, 43, 80) employ *Allium* in studies of the structure and behavior of the chromonemata, which are particularly clear in many members of the Liliaceae. Bělăr (3) illustrates his text with a sequence in *Allium cepa*, and Heitz has investigated the nucleolus

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Since submitting this article and preparing a sequel to it, Mr. Vanderlyn has died. Any inquiry concerning the article should be sent to Dr. Jean MacCreight, Biological Abstracts. [Ed..]

(31) and chromocenters (33) in this species. Levan (45–53) has made a thorough study of chromosome number and morphology in the genus, and an extensive review of this literature is contained in a paper of 1933 (42).

As recently stated (77), every theory in the history of cytology which has attempted to reduce karyokinesis to the operation of simple or unit forces has failed to explain all the known phenomena. For this reason the details of mitosis should be considered not as a succession of isolated pictures, but as an integrated process occurring in time; not as the operation of forces in a field that is in any way homogeneous, but rather as the interplay of numerous forces involving many cell parts, intricately constructed and often intercontained.

The present paper is a review of the important data on onion-root tip mitosis, into which a certain number of original observations have been integrated in order to give a more unified and consistent four-dimensional picture of the process. The base of these findings is broadened to a picture of cell structure and division in many forms.

Darlington (12), in his discussion of cell mechanics, divides the analysis of the forces operating in karyokinesis into data of external mechanics ("movements of individual chromosomes in relation to each other and to bodies outside of them") and of internal mechanics, *i.e.*, the mitotic cycle as a sequence of coiling and uncoiling of chromonemata. A true picture of the mitotic process as a whole should relate the spiralization cycle from telophase to telophase within the chromosomes with chromosomal movements through the nucleus during the same period. Omission of such correlation in a form serving as a textbook model for mitosis obviously constitutes a serious gap.

The spiralization cycle has been described for *Allium cepa* (1, 2, 42), but the intra-nuclear movements of the chromosomes have never been fully investigated. The fact is, they are not accounted for in thorough detail through an entire mitotic cycle in any one form, plant or animal, with the exception of a paper by Koerperich (40) which deals with three species of plants, selected because they contain both large and small chromosome complements within the same genome.

The present paper attempts to parallel in *Allium* Koerperich's results for *Northoscordum fragans* and *Eucomis undulata* (Liliaceae) and *Beschorneria yuccoides* (Amaryllidae), and to repeat with modifications the spiralization cycle as described for *Allium* (1, 2, 42). Both series of events are treated as a single process. The history of the nucleolus and the present status of our knowledge concerning its structure, chemical composition and function are also briefly traced.

The original observations were made on standard preparations of the onion-root material, some my own, some obtained from collections at the University of Pennsylvania and Columbia University. Of especial value have been Feulgen preparations by Mr. Rafalko of the University of Pennsylvania, and Flemming iron-haematoxylin preparations by my former assistant, Miss Margaret Yarnall. The nature of centromeres as regions of constant connection with the nuclear membrane, and their collaboration with the membrane in the formation of the metaphase plate, the meaning of the rotation of the nucleus in the mitotic process, together with the overall correlation of the spiralization cycle with intranuclear chromosome movements, constitute the more important of my original contributions. They serve somewhat as catalysts, serving to organize and activate a number of unorganized but potentially useful facts.

ROOT-TIP HISTOLOGY

A proper understanding of the somatic cell cycle in the root tip requires some knowledge of its histology, as the appearance of the nuclei and of the division figures varies in the different types of cells. The various regions are best identified from a longitudinal section made through the core of the root. Theoretically speaking, almost all the cells are meristematic, *i.e.*, actively dividing. Many of them, however, are in various stages of differentiation, depending on their location.

At the curved tip, which terminates the cylindrical root, all cells are primary, undifferentiated meristem. Covering the tip is a cap of tissue in which the cells are polyhedral, the nuclei irregular and compact, and the cytoplasm greatly reduced. Above the band of undifferentiated tissue at the tip lie three zones of early differentiation: (*a*) plerome which will give rise to the vascular ducts, (*b*) periblem which will form the cortex, and (*c*) dermatogen, destined to form the epidermis. The plerome is the central cylinder of cells in which longitudinal divisions predominate. Its center is occupied by one or two columns of giant cells with large nuclei and proportionally large nucleoli. These will form xylem. Surrounding this region there are several columns of narrow, elongate cells with correspondingly elongate nuclei, their two or three nucleoli generally arranged in a line parallel with the long axis of the cell. Here the division figures are very irregular in appearance, due to the narrow space in which the chromosomes are crowded. Lateral to these columns plerome shades off into undifferentiated meristem, with no distinguishable boundary between.

Periblem cells are primary, undifferentiated meristem, usually rectangular in cross section, and with typical longitudinal division, although transverse division occasionally occurs. The transition of meristem into a cortex begins higher up in the root tip than the level at which differentiation begins in the plerome, so that periblem cells are the best source of meristematic division. The outer zone of periblem is a tightly packed layer of cells with oval nuclei, the majority being oriented with the long axes of their nuclei along the radius of the cross section.

Dermatogen is a single, protective layer, its cells resembling those of the root tip, but more elongate.

Thus the primary undifferentiated meristem at the extreme tip is continually transforming into four regions: the central plerome, the periblem, dermatogen, and the root cap or calyptogen. The transition of meristem into extremely differentiated permanent tissue is marked by vacuolization of the cytoplasm and migration of the nucleus to a position in the peripheral layer of the cytoplasm, closely applied to the cell membrane (62). Except where otherwise designated, the figures in this paper are of the typically rectangular meristematic cells of the periblem.

THE MITOTIC CYCLE

Since the primary concern of this paper is with the movements of the nuclear components during mitosis, their finer structure will not be discussed except where it is related to these movements. Extant theories of the structure of the resting nucleus all reflect the older debate over the nature of protoplasm itself, *i.e.*, whether it is fundamentally alveolar, reticular, granular or filar. Some cytologists reflect the old fibrillar viewpoint, since they believe reticular and granular structures are actually the optical effects of many minor coils spun out from a more compact state and filling the nucleus.

It is my belief that the small size of resting stage alveoles, reticules and gyres precludes settlement of this point by observation alone. Intrachromosomal pictures exhibit similar variations with treatment, the same area under different conditions and fixatives showing gyres of coils, alveoles or granules with interconnecting lines. Some authors (44, 57) see a causal and developmental connection between these different types of structure.

Although the main and novel contribution of this paper is one of intranuclear movements, we are secondarily interested in integrating and bringing to an elementary level a body of important data still unincorporated in elementary texts and therefore unavailable to beginning students. These data are as follows:

(*a*) Though the general student of biology is acquainted with the fact that chromosomes in some way maintain their individuality through the interphase, it is not so generally recognized that through this period they also retain their telophase polarity. Use of this point would greatly simplify textbook and blackboard illustrations of mitosis. In a later paper, moreover, I shall assemble the at present unorganized evidence that the polarity of the cell is significantly reflected in the structural organization of the chromosomes themselves.

(*b*) In connection with the above, the location of the nucleoli on definite regions of definite chromosomes, and the relative constancy of their position with respect to the latitudinal axis of the cell (owing to chromosome polarization), are important clarifying points which have not yet reached the level of the elementary text. Again, despite general ignorance concerning its true function, a great deal is known of the topography and structural relations of the nucleolus which would aid the student in formulating a correct conception of the generalized cell. This, too, has not yet filtered down to the elementary level.

(*c*) With reference to plant nuclei in particular, the established fact (11, 16–18) has not become widely known that although transitions exist, there are two main types. The first has small chromosomes, and a clear homogeneous nucleus in resting stage, and the chromosomes remain in contact with the nuclear membrane and pycnotic for a considerable portion of their length. In this state they are known as "euchromocenters" or "prochromosomes". The second type has long chromosomes, and the resting stage is reticulate and studded with small granules which function as centers of condensation in chromosome formation. These granules are known as "chromocenters".

Much confusion exists in the literature concerning chromocenters. By definition they are chromosomes or parts of chromosomes which remain in the resting nucleus (25). Even in forms where they normally occur, however, there are resting nuclei in which they are entirely absent. Also, temporary chromocenters have been described which are formed when the cell is stimulated (63). Wilson's text (86) does not include them in its glossary, although Tischler's plant cytology (83) devotes several pages to the subject. Their very thorough investigation by Heitz (33) is made from a purely morphological and cytogenetic standpoint, and he does not mention the somewhat extensive experimental and deductive evidence concerning their probable function. This has recently been reviewed by Milovidov (63). Finally, there is no complete agreement as to just which chromatin granules are to be considered chromocenters.

As Heitz (30) recognized, the problem of their form and structure is linked with the problem of heterochromatin, which is at present in a state of complete confusion. There is a multiplicity of definitions of heterochromatin, no agreement on just which structures in the nucleus are heterochromatic, and a bewildering array of properties and functions have been ascribed to it. Now, as is frequently the case, the most controversial frontiers of any discipline often relate to and serve to focus upon its basic and essential problems. The current discussion of the nature of heterochromatin cuts across almost all aspects of cell structure and division, cytological, genetic and biochemical. It is my belief that much of this confusion has arisen from a failure to consider its allocation in the cell; and this allocation is one of the missing links which it is the task of the present paper to supply. In a subsequent paper, to appear in this journal, I shall review the problem of heterochromatin, rearranging its manifold and often conflicting data to a more consistent and intelligible order in the new framework, and attempting to arrive at a better working definition of what heterochromatin is, and a more plausible theory concerning its function. For the present I shall employ Heitz's original definition of heterochromatin as that chromosome substance which retains its maximum metaphase condensation, in contrast to euchromatin, which undergoes the customary telophase dispersion (29). Chromocenters are thus obviously, by this definition, heterochromatic.

In *Allium cepa,* despite the fact that many authors have pictured its resting nucleus free from chromocenters, Heitz (33) succeeded in demonstrating them with a variety of treatments. I have observed them in all haematoxylin and Feulgen preparations, although they are not so sharply differentiated in the latter. The Feulgen reaction does not demonstrate any essential difference between hetero- and euchromatin (36). With other methods their appearance is more erratic. In general, where the chromosomes are not strongly differentiated, the chromocenters are absent. When present they appear consistently in all the root tip tissues and are most easily studied in the giant water-duct cells. This implies their persistence through the metabolic stages. As will be seen, there is evidence for their participation in cell metabolism.

Fig. 1 depicts a meristematic cell in the zone of differentiation. The nucleus happens to be slightly rotated upon its axis, a phenomenon which will be explained later. The two nucleoli are seen as large haematoxylin-chromatic spheres, and a line drawn between them establishes the latitudinal axis. A line perpendicular to the latitudinal axis defines the longitudinal axis passing through the poles.

The upper or proximal pole thus lies slightly to the upper left, and the lower, or distal pole, slightly to the lower right of the figure. The upper pole is distinguished by a peripherally disposed irregular constellation of proximal chromocenters. The lower pole is marked by smaller, pronouncedly more spherical and sharply delineated distal chromocenters.

The proximal chromocenters are longer and more or less parallel to the longitudinal axis. They tend to fuse in what Heitz calls a "nuclear cap" (Kappenkern). As Fig. 5 shows, these regions are the heterochromatic proximal and distal ends of the chromosomes. Those of the proximal region are similar to prochromosomes in that they extend a distance along the length of the chromosome arm and may be considered as a transition toward the euchromocentric type. In haematoxylin preparations both proximal and distal chromocenters exhibit curves and spirals, and give the impressions of being peripherally located in vacuoles which are in some way connected with the general interphase vacuolization of the nucleus.

Tangent to, or a short distance from, the nucleoli, there are conspicuous, large, rotund granules. Heitz identifies these as the trabants or satellites of the two nucleolus-bearing chromosomes which persist through the interphase ((31), Fig. 5 for *Crepis* and *Lactuca*). Similar structures in *Zea,* however, are described (67) as the nucleolus-organizing region. Their true identity is hence undetermined. From the situation in the lower nucleolus of my Fig. 1, the possibility that both trabant and nucleolus-organizer may be present must be considered.

Other granules, some round, some irregular, are distributed on the lateral surface of the nucleus. Still others, of a size smaller than either the polar or distal chromocenters, are scattered throughout the nucleus. The proximal chromocenters are largest, the distal and lateral ones smaller, and the intranuclear granules, other than those associated with the nucleolus, are smallest. As is suggested in my Figure 8, and in Heitz's Figs. b and m (33), there is a possibility that the smaller granules are due to the persistence in interphase of the chromatin which ranged along the periphery of the chromosomal matrix, and that these are in some way associated with the chromonemal gyres. Also, they may be in whole or in part adventitious or temporary chromocenters which are neither permanent structures nor associated with any known feature of chromosome morphology.

There are thus two differentials in the amount and distribution of chromatin, an antero-posterior differential between proximal and distal chromocenters, and an extero-interior differential between the peripheral chromocenters and the interiorally located granules. The latter gradient reverses itself at what may be considered an innermost region, the neighborhood of the nucleolus, where the trabant chromocenters may be of conspicuous size. The antero-posterior differential reflects the polarity of the cell, since where daughter cells remain closely associated and in a similar stage of development at post-telophase, it can clearly be seen that the proximal chromocenters are the centromere regions, and are polarized at the active or division pole of the cell. In the succeeding paper I shall offer an explanation of these gradients in terms of cell polarity and cell dynamics and what is known of the biochemistry of nucleic acid.

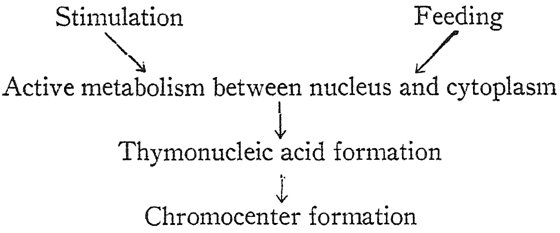
Heitz's definition and classification of chromocenters should now be reviewed (32). He regards chromocenters as definitely located chromosome regions, more strongly stained than the remaining sections, and visible principally in prophase and telophase. He classifies them as

A. Telophasogenic heterochromatin: Permanent heterochromatin.
  a. Heteropycnochromocenters. To this type belong the distal chromocenters of *Allium.*
  b. Microchromosomic chromocenters. These are the prochromosomes or euchromocenters of the short chromosomed plants. They differ from the above in that almost all the chromosomes of the set are involved, and also in the constancy of their location at the proximal pole of the cell. They may form fused chromocenters or polar caps (Sammelchromocentren).
  c. Polar cap chromocenters (Kappenchromocentren). These are chromocenters which, while not prochromosomes, lie on the nuclear membrane at one pole and fuse to form polar caps. The proximal chromocenters of *Allium* appear to belong in or somewhere near this category.
  d. Trabantochromocenters. These are spherical trabants or satellites found in many plants which form strongly staining chromocenters in the resting nucleus, and which are often indistinguishable from other similar spherical bodies. In *Allium* they may be identified with ease (Fig. 1). That nucleolus-organizers may also be present in this region, and hence indistinguishable from trabants proper, should be borne in mind.

B. Karyogenes, or temporary chromocenters.

Considerable literature exists which indicates that these may be produced by a number of stimuli-feeding, wounding, pressure, and organic and inorganic agents. Milovidov (63) has shown that these chromocenters are Feulgen-positive. Several authors quoted by him are of the opinion that this phenomenon is indicative in some way of a transfer of substance between the cytoplasm and the chromocenter regions. Others point out the resemblance between the appearance of these chromocenters and the events of chromosome formation at prophase. For this reason some of the chromocenters described in the above literature are probably of the permanent type.

Since these are Feulgen-positive, they may be considered heterochromatin. Milovidov postulates the following scheme for the situation in *Drosera:*


Stimulation
Feeding
Active metabolism between nucleus and cytoplasm
Thymonucleic acid formation
Chromocenter formation

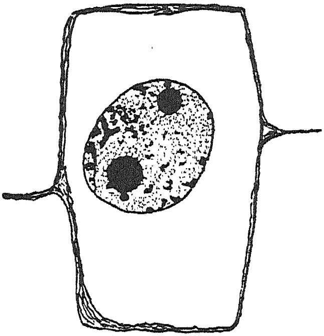
To the last item should be added the phrase, "or enhancement of the nucleic acid content of permanent chromocenters", since the telophasogenic heterochromatin may also be affected. The interesting and inclusive point is that these peripheral chromocenters, permanent or temporary, appear to be linked in some way with cell metabolism. This leads us to the next issue.

Heitz makes a great deal of what he terms "equivalent heterochromaticity"—the tendency for homologous regions of non-homologous chromosomes to be heterochromatic (30). This, he believes, shows them to be under genetic control. From my point of view, however, considering their topography, it seems patent that epigenetic factors may be involved, *i.e.,* heterochromaticity may be due to the relationships of the chromosome with its external environment. In *Allium* the regions where the chromosome is in contact with the nuclear and nucleolar membranes are most conspicuously heterochromatic. These are the proximal, distal and trabantochromocenters. This is apparently also the case in *Zea mays*, in the attenuated pachytene of which we have accurate identification of the heterochromatic areas. In this species there are (*a*) the nucleolus-organizing region of chromosome 6, (*b*) the knobs, (*c*) the regions adjacent to the centromeres, and (*d*) the chromomeres (67). We may ignore the last category, since if chromomeres are considered heterochromatic, and heterochromatin is considered genetically inert, then we are involved in a hopeless contradiction, as the chromomeres are at present believed to be the gene-bearing regions.

In addition to the above, some strains of maize have totally heterochromatic chromosomes in varying number, the B set. Morgan, in examining the whole resting nucleus of the maize root-tip,

Mitotic Stages in *Allium*

1. Meristem cell in resting or metabolic stage; Bovin's, iron haematoxylin. *2.* Meristem cell in middle prophase, same. *3.* Two daughter cells in late telophase. Second chromosome from the left in both cells shows nucleogenic region and re-forming nucleolus. Flemming's, safranin fast-green. *4.* Chromosome in middle prophase. Bovin's, iron haematoxylin. *5.* Metaphase chromosome in last stages of the unwinding of the sister chromatids. Centromere a chromatic projection. Bovin's, iron haematoxylin. *6.* Late prophase chromosome. Right arm bears what is probably a satellite, or translocation. Bovin's, iron haematoxylin. *7.* Same as 5, but with the centromere as an achromatic constriction. Bovin's, iron haematoxylin. *8.* Early prophase, showing polar cap (Kappenkern) consisting of heterochromatic proximal regions and distal chromocenters which are the distal regions of chromosomes where they are in contact with the nucleolar membrane. Flemming's, iron haematoxylin. *9.* Diagrammatic representation of a polar view of middle prophase (Fig. 2). Outer ellipse of dotted line represents the polar cap, inner circle, the apical region of the cell; and transverse line, the future equatorial plate. (See text for explanation.) *10.* Metaphase chromosome in act of separation. Bovin's, iron haematoxylin. *11.* Anaphase chromosome with outline suggesting the coils of the next mitotic cycle. Bovin's, iron haematoxylin. *12.* Emergence of chromonema in early prophase. Flemming's, iron haematoxylin. *13.* Gyres of emerging prophase chromonema. Flemming's, iron haematoxylin. *14.* Telophase-dissolution of chromosome arm. Flemming's, iron haematoxylin. *15.* Nucleolus of large xylem cell. Chromatin elements at periphery, which are in actuality continuous with the Feulgen-positive elements of the nucleus. Vacuole containing inclusion in rapid Brownian movement. Feulgen preparation imbedded in clarite, two years old. *16.* Chromosome early to middle prophase showing gyres of coils and attachment to nuclear membrane. Flemming's, iron haematoxylin. *17.* Diagram of two daughter cells in interphase, which have commenced rotation in a corresponding direction. Polar chromocenters indicated. *18.* Emerging coils in telophase, showing diamond lattice appearance and association with periphery of matrix. Flemming's, safranin. Figures 9 and 17 are diagrammatic. All others are drawn with a camera lucida at a magnification of 950 diameters. In reproduction the original drawings have been reduced to approximately one half.



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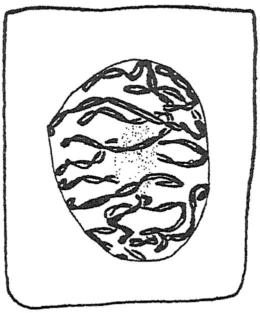

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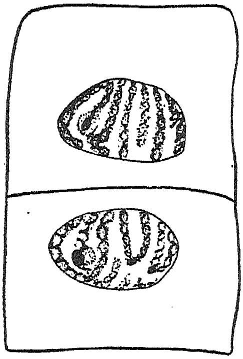

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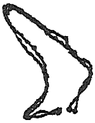

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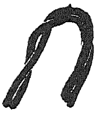

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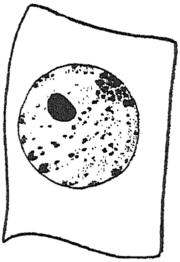

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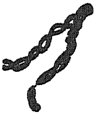

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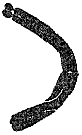

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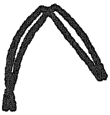

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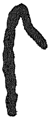

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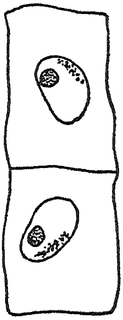

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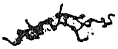

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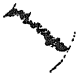

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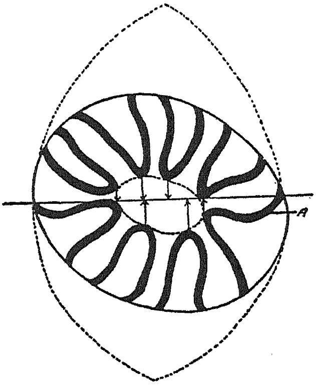

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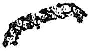

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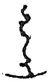

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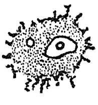

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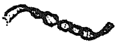

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found the chromatic material located in two places, (*a*) next to the nucleolus (he designates these as nucleolus-organizers, although they do not differ in appearance from the trabantochromocenters of Heitz), and (*b*) around the nuclear periphery ((67) figure 1D). In the resting stage such chromatic regions would be heterochromatin. Thus the heterochromatin is located, as in *Allium,* principally at the nuclear and nucleolar membrane surfaces.

From the high degree of correlation which he found between the number and size of the pachytene knobs and the number and size of chromocenters in root tip interphase, Morgan postulated that the knobs were actually chromocenters. Through the courtesy of Dr. Rhoades at Columbia University, I have checked with the original photographs for the Morgan papers, and these seem to indicate that the chromocenters are peripherally located. Careful study of this situation in *Zea* is highly desirable, as this is the one plant form in which the chromosomes are genetically mapped.

From Kaufmann's work (39) on somatic mitoses in *Drosophila melanogaster,* the heterochromatic sex chromosomes are in contact with the nucleolus. Sometimes (his Fig. 2 and 40) the sex chromosome appears conspicuously heterochromatic over just that area which is applied against the nucleolus. In *D. melanogaster* and *D. funebris* (34, 35) the nucleolus-bearing constriction of the X-chromosome in prophase lies in the heterochromatic, proximal portion of the X. The other heterochromatic region in *Drosophila* is in the centromere region of the large V-chromosomes. Heterochromatic sections lie on both sides of the achromatic constrictions. If, as may be predicted on the basis of the present findings in *Allium,* the centromere region remains in a peripheral location throughout the mitotic cycle, these regions are likewise regions of proximity to the nuclear membrane. Critical investigation of these points in *Drosophila* material seems indicated.

In the subsequent paper I shall give further evidence that chromocenters, in their nature as heterochromatin, are in some way concerned with an exchange of energy or substance across the nuclear membrane. The idea that heterochromatin is a genetically inert region seems diametrically opposed to this. This idea too stands in need of careful reconsideration.

The examples in Milovidov's paper were of the effect of stimulation on chromocenters as increasing their staining capacity and presumably their nucleic acid content. A paper entitled "Nucleic acid starvation of chromosomes in *Trillium*" (14) is of interest in showing the action of cold in producing the reverse effect, *i.e.,* a diminution of nucleic acid content. This will be discussed here because its interpretation of the causes of this phenomenon is an outstanding example of how obvious epigenetic factors may be overlooked in favor of less substantiated and even less plausible genetic explanations.

The authors find for *Paris* and *Trillium* that "under ordinary conditions their chromatids have a uniform staining capacity and . . . a uniform diameter, apart from the usual constrictions at the centromere and at points of nucleolus formation". Following subjection to low temperatures at a previous stage of the mitotic cycle, "certain segments in each chromosome are reduced to half the standard diameter of the chromatid and appear understained from late prophase until anaphase. They remain half their usual length. This difference is clear not only with gentian violet staining but also with the specific Feulgen reaction". The authors conclude that these segments are "constant in size and position throughout the root tips of the individual" and "are undercharged with nucleic acids".

The next point is a critical one. They further establish that these differential regions correspond in number and size with the chromocenters of the resting nucleus. "The chromocenters, especially in *T. erectum,* are darkly stained and highly refractive, so that the larger ones resemble metaphase chromosomes". "This comparison" they conclude "leaves no doubt that the over-nucleated chromocenters of the resting stage are in fact the undernucleated differential segments of metaphase. They are the heterochromatic part of the chromosomes. . . . The undercharged metaphase segments thus arise at low temperatures from overcharged resting stage segments".

In this connection one salient feature in nuclear topography was overlooked by these authors, as it has been by most writers whose perspective is primarily genetic, namely, that these chromocenters are peripheral in nature, and with it the obvious corollary that they may represent regions of the chromosomes which would be most sensitive to environmental influences acting upon the cytoplasm. It seems more simple, more plausible, to assume that low temperature directly affects these sensitive regions, disturbing their normal nucleic acid cycle, till they show undercondensation in the subsequent stage, than to postulate, as do Darlington and La Cour, that that "abnormal cycle . . . is specific to the gene or group of genes comprising them". Or again, as stated in their conclusion, that "The undercharging of metaphase . . . seems to be conditioned by (*a*) a lower supply of nucleic acid in the nucleus, and (*b*) a lower competitive strength or demand of the inert genes for nucleic acid in the presence of an insufficient supply".

The concept of "inert genes" will be discussed under the topic of heterochromatin in the later paper. It should be noted, however, that since no chromosome maps exist for *Paris* and *Trillium,* the inertness of these regions is purely inferential.

THE NUCLEOLUS IN GENERAL

Morphology. In his brief but excellent historical account of the discovery of the relationship between the nucleolus and chromosomes, Gates (23) comments: "The last decade has seen a striking advance in our understanding of the origin of the nucleolus in the mitotic cycle and its relation to the chromosomes. In tracing . . . this development, which represents a major discovery in relation to the organization of the nucleus as a whole, we may treat the subject as an instance of the methodology of science; for it illustrates how a slight increment of knowledge may lead to a complete change or reversal in the point of view". As a result of the combined work of several investigators (23, 31, 59, 64, 65), the following facts are now definitely established regarding the morphology of the nucleolus in higher plants:

a. The nucleoli of two sister cells correspond in size, number and position.

b. Although their position on the latitudinal axis, *i.e.*, that on the equatorial plane of division, is variable from cell to cell, in most cases their height, *i.e.*, their position on the longitudinal axis, is fixed.

c. In many plants the nucleoli arise in telophase on definite chromosomes—the SAT (sine acido thymonucleinico)-chromosomes, as they have been designated by Heitz—which are often characterized by a trabant or satellite separated from the body of the chromosome by an achromatic secondary constriction or satellite stalk. The truth of (a) above is thus due to the constancy in number of these chromosomes and to their corresponding positions in sister chromosomes in anaphase and telophase; of (b) to the constancy in position of the secondary constriction along the SAT-chromosome length.

d. 1. The size of the nucleolus and the length of the secondary constriction usually correspond.

2. When two SAT-chromosomes are present in the complement, distinctive size differences of nucleoli exist, correlated with differential functional activities of the two.

e. Fusion of the nucleoli may occur early in telophase.

f. Because there is often but one SAT-chromosome to the monoploid set, the number of nucleoli in the cell is sometimes an index of its ploidy, as was first noted by Parmenter (68). There are, however, many cases where this correspondence is lacking, including (e) above.

g. In *Zea mays*, and possibly in other forms, the nucleolus is formed by a special heterochromatic nucleolus-organizing region, directly adjacent to the stalk of the satellite. In this case, the satellite stalk appears to owe its origin to a stretching across the nucleolar surface rather than the nucleolus owing its origin to the satellite stalk.

h. Under certain circumstances chromosomes other than the SAT-chromosomes of a complex produce nucleoli. It has been reported (32) that in *Vicia* a non-SAT-chromosome lagging upon the spindle and transforming into a karyomere also produced its own nucleolus. In the absence of the most active moiety of the nucleolus-organizer there appears in the nucleus a constellation of many nucleoli, born on many chromosomes (59). Conversely, it should be noted that there are cases of secondary constrictions which bear no nucleoli at all, for example, that in the left arm of the second chromosome in *Drosophila melanogaster* (39).

The above established findings apply only to that type of cell with a typical reticulate resting stage. In the prochromosome or euchromatic types, with a homogeneous nuclear cavity in this stage, the nucleolar history is different and will be described as the mitotic cycle of this type is recounted contrapuntally to that of *Allium*. Of the findings of Doutreligne and Zeeuw (15, 87) on this subject, Geitler's comment should be kept in mind: "Diese Verhalten ergibt aber keine Widerlung der Beobachtung von Heitz . . . sondern bildet eine Erganzung derselben" (25).

From this point on we enter the controversial frontiers. Heitz believed the achromatic constrictions produced the nucleolus. As matter of fact, visible heterochromatic nucleolus-organizing regions have been reported in only a few cases. Again, Resende (71), in attempting to establish the ubiquity of SAT-chromosomes among flowering plants, encountered one exception: "Bei *Trillium longiflorum* wurden ausser den Nucleonen, die sich an den SAT chromosomen kondensieren, solche beobachtet die in keiner Verbindung mit irgendwelchen Bildungen stehen". Conversely, in the Aloinae he found secondary constrictions which had no nucleolus. Later Matsuura (60), investigating *Trillium* and *Paris,* came to the conclusion that the nucleolar chromosomes of plants may be divided into two types, *viz.:*

(*a*) Interstitial type, in which the nucleoli develop at the constricted part of the SAT-chromosome, and
(*b*) Terminal type, in which the nucleoli develop at definite ends of specific chromosomes.

In the second case "there is neither a heterochromatic body nor a region at the end of these chromosomes to which the nucleoli are attached. Sometimes, however, fine connecting strands are visible in these regions . . . considered to be of nucleolar origin, not part of the chromosome proper". It should be remarked, though, that in liverworts (55) and in *Narcissus* (19) heterochromatic distal extremities were noted to which the nucleoli were attached, on non-satellited chromosomes.

Fernandes's findings (19) are of especial interest. He describes for *Narcissus* two types of satellites, (*a*) heterochromatic, having the characteristics of the nucleolar organizing region, which he terms the "nucleogenic region," and (*b*) euchromatic satellites, which lack these characters. His interpretation is that the satellites of the first type arise when the nucleogenic region is situated at the extremity of the chromosome. When the point of greatest activity is found at the exact extremity, no satellite will be formed. When it is in an intermediary region, a satellite will appear, whose stalk will be proportional to the distance of the region from the telomere. If the active point is located at the proximal point of the nucleogenic region, the entire region becomes a satellite.

Euchromatic satellites presumably have their origin when the nucleogenic regions are located elsewhere than at the telomeres.

The satellites may be more or less attenuated, depending on the distance of the nucleogenic region from the telomere. Fernandes believes that the satellites of *Zea mays* are of this type. The validity of the last interpretation especially must await a three dimensional analysis of the relations of satellites with the nuclear membrane. But the existence in *Narcissus* of two heterochromatic regions may explain the two trabantochromocenters found in the lower nucleolus of my Fig. 1. One may be the true satellite, and the smaller may be the nucleogenic region.

Matsuura (60) postulates two general principles governing the nucleolus-chromosome relationship: "First, every chromosome can be referred to as 'nucleolar chromosomes' in the sense that it can produce nucleoli under certain specified circumstances. This is clearly indicated by the fact that when each of the chromosomes of a complement happens to form a micronucleus, as caused by the irregularity in the second division of 'precocious' pollen-mother cells in *Trillium,* each micronucleus is provided with at least one small nucleolus". Matsuura cites in this connection the somewhat similar evidence of Heitz and McClintock mentioned above. He continues, "Secondly, there is usually a differential rate in the capacity for nucleolus-organizing activity of chromosomes within a complement, thus resulting in the usual occurrence that particular chromosomes alone are apparently related to the formation of nucleoli. These particular chromosomes have greater, consequently more rapid, rate of functional activity than the rest".

Physical, chemical and physiological properties. Of the three types of nucleoli designated by Wilson (p. 91, 3rd edition), the karyosomes, or chromatin nucleoli, the plasmosomes and the amphinucleoli, we are dealing only with plasmosomes or true nucleoli. Many of Wilson's criteria for distinguishing these bodies no longer apply. The idea that the plasmosomes are "not directly connected with the framework of the nucleus" has been outmoded by the facts listed above. The view that "they are devoid of a distinct limiting membrane" is for the most part discarded. The basic distinction that karyosomes contribute directly to the formation of the chromosomes whereas plasmosomes do not is meaningless when we consider that almost all the structures designated by Wilson as examples of the former have since turned out not to be nucleoli at all. The chromatin nucleoli are simply monosomes and other heteropycnotic chromosomes or parts of chromosomes. The Feulgen technique reveals net-knots as any one of a variety of structures—chromomeres, chromocenters or artefacts produced by the haematoxylin method. Wilson himself states that what were at the time called karyosomes in the Protozoa are probably not comparable to those described for the Metazoa. Wilson's example of the basichromatic nucleus in *Spirogyra* also goes by the board, as Geitler (26) has shown that this is Feulgen-negative, and not in any way karyosomal. The whole category has probably become invalid with the passage of time, though very useful in its day, and with it the amphinucleoli which were supposed to consist of both types. The term "nucleolus" should certainly be limited nowadays to the plasmosome proper, as it is in most recent papers.

In the following are listed only the properties of plasmosomes which are more or less accepted, or at least unchallenged. So scanty is our fund of information under this head that we must draw on both plant and animal kingdoms. For further details two important references may be consulted (23, 66). With regard to the function of the plasmosome, it is proverbial that critical information is in inverse proportion to the volume of the literature.

I Physical properties.

a. The nucleolus has a refractive index different from that of the karyolymph. In the living condition it is usually seen as a highly refractive sphere.

b. Although typically spherical it has been described as ameboid in different species, in different stages and under different conditions.

c. Centrifuging shows it to be liquid, of greater density than the karyolymph.

d. The fact that it may persist in the cytoplasm many days after its expulsion by centrifuge (12) shows that its limiting membrane must possess considerable integrity. (See also the evidence from the Brownian movement of the nucleolinus in *Allium* presented below.)

e. Its contents are not homogeneous. The nucleolus may contain vacuoles and crystals. A frequent picture is one of dichotomy of nucleolar substance, the discontinuous elements contained within the continuous as one or several vacuoles, or as several refractile crystalline bodies; vacuoles and inclusions sometimes co-exist. These nucleolar inclusions have been called nucleolini. (See *Allium* below.)

f. In electrophoresis the nucleolus has been shown to move toward the positive pole, suggesting that its membrane surface bears a negative charge.

II Chemical properties.

a. Staining affinities.

For an account of the pitfalls of error and general uncertainties on this subject the two papers by Zirkle (88, 89) should be consulted. The following are among the generally admitted facts:

(1) Nucleoli, in the greater number of cases, plant or animal, stain blue to black with Heidenhain iron haematoxylin technique, as do the heterochromatic regions of the chromosomes, at the time the euchromatic regions exhibit minimum basichromaticity. They may show a loss of basichromaticity *pari-passu* with its incrementation in the euchromatic regions through prophase to metaphase.

(2) In general, they show special affinity for acid dyes, and in this respect resemble the cytoplasm and the matrix of the chromosome.

(3) They are, with few exceptions, Feulgen-negative. The reported exceptions consist for the most part of Feulgen-positive granules scattered through the nucleolus, or concentrations of Feulgen-positive substance at its periphery. This is indication that the nucleolus does not ordinarily contain desoxyribose nucleic acid.

(4) That the contradictory results in the staining reactions of the nucleolus may perhaps be attributed to variations in the chemical nature of its contents is supported by cases such as the nucleoli of mouse and frog tissues (5). After special fixation and staining with methyl blue these nucleoli show two types, one staining red and one staining blue; since the latter disappeared on starvation and reappeared on feeding, it was suggested this type might consist of reserve substances.

b. Ultra-violet absorption analysis.

The work of Caspersson and his associates in this new frontier has been summarized and discussed in a number of recent papers (13, 75, 76, 81,). Using ultra-violet microscopy in combination with other methods, Caspersson analysed the salivary gland chromosomes of *Drosophila*. On the basis of differentials in absorption he was able to distinguish between the nucleic acids and proteins, and also to differentiate between histone and globulin type proteins. Heterochromatin and the nucleolus have high content of histone type proteins; the euchromatin intercalating the heterochromatic areas contains globulin type proteins.

This similarity in high histone content between heterochromatin and nucleolus "suggests that one secretes the material of the other, or we might suppose, some precursor of it" (13). I also suggest that there may be some connection here between the common basichromaticity of nucleoli and heterochromatin in iron haematoxylin treatment.

As we noted above, the Feulgen-negative nature of the nucleolus indicates that it contains no desoxyribose (thymo-) nucleic acid. The ultra-violet absorption spectrum does reveal the presence of ribose nucleic acid. Pollister and Mirsky (70) propose the term "plasmonucleic acid" for the latter, because it is found in the plasmosome and in the cytoplasm; for desoxyribose nucleic acid they suggest the term "chromonucleic acid" because it is confined to nuclear chromatin. These terms have the advantage of simplicity and correspondence with the topographical facts, and will hereafter be employed in this paper as more useful and appropriate to the cytologist than the somewhat unwieldy chemical names.

III Physiology.

Most of the examples of responsiveness of nucleoli to changes within and without the cell involve alterations in size. Nucleolar size is variable with the tissue. Van Camp (84) found for *Clivia* that the sum of the cubes of the nucleolar diameters was equivalent in neighboring cells of a tissue, and that nucleolar material is greater in meristematic than in differentiated tissue, "et par consequent . . . il existe une relation de fait entre la faculté caryocinetique et la quantité de matière". And also "que la matière nucleolaire est susceptible de s'élaborer suivant la destinée et la rôle d'une cellule . . .". The following are some noteworthy examples of size change in response to varying conditions and stimuli:

1. In *Dahlia* nucleoli were reported present in guard cells when they were open, and absent when they were closed; there was thus a diurnal cycle in their appearance and disappearance (85).
2. Fischer (21) found a difference in mean volume of 20% to 50% between afternoon and morning, in the nucleoli of the leaf cells of *Bryophyllum*. When leaves were placed in the dark they underwent considerable diminution in size. When leaf fragments were grown in sugar solution the nucleoli were larger. He concluded that the diurnal variation was actually correlated with carbohydrate metabolism, the afternoon, when nucleoli were largest, being the time of greatest carbohydrate storage, whereas in the morning, when such storage was at a minimum, they were smallest.
3. Possible connection with (2) above may exist in the observation that in regeneration of mosses and liverworts, as the chloroplasts get smaller the nuclei and nucleoli become larger (28).
4. Wounding has a definite effect upon the nucleoli in leaves of *Peperomia* (21). Nuclei near the wound edge enlarged, as did the nucleoli. In regeneration experiments with *Lumbricus* nucleoli were found to be larger and double 11 or 12 segments from the wound. In general, all cells of regenerating tissue had large nucleoli. From this the conclusion was drawn that the amount of nucleolar material in the cell is in some way an index of its nuclear activity (73).
5. Again, Fischer found that if cell division were inhibited, the nucleolus grew larger. The same was found by Conklin in *Crepidula* (8). He stated that nucleolar size depended upon cell size and also upon the length of the resting period, cells with the longest resting period having the largest nucleoli. In cells in which division had been inhibited by hypertonic salt solutions the nucleoli were much larger than in normal eggs.

6. Another series of findings concerns changes in the nucleolus which occur in animal neoplasms.
   (*a*) Pianase (69) first reported that the nucleolus of tumor cells was larger than in the normal. Ludford (56) believed that large nucleoli were characteristic of many tumors.
   (*b*) The plasmosome has been reported as hypertrophied and irregular in all neoplasms as contrasted to the normal spherical and compact nucleoli of adjacent stroma (37).
   (*c*) An average increase in the nucleoli directly proportional to the malignancy of sarcoma tissue was reported by Sato who advanced the hypothesis that nucleoli, through their numbers, chemical composition and situation in the nucleus, are responsible for the abnormal metabolism of malignant cell proliferation (72).
   (*d*) In hypophyseal adenomas the nucleo-nucleolar ratio of simple adenomas has been found to be greater and more variable than in normal glands (22).
   (*e*) MacCarty (58) stated as a general rule that "the nucleolus is much larger in proportion to the size of the nucleus in all malignant cells, regardless of the type or origin of the neoplasm", and went so far as to claim this as an identifying characteristic of malignant tissues. This was objected to by others, notably Cowdry and Paletta (10) who found that in mouse epidermis neoplasms, while it was true that most of the specimens showed abnormally large nucleoli, this was not without exception.
   (*f*) Koller (41) discusses the rôle of the nucleolus in malignancy. He errs, however, in considering heterochromatic regions as nucleoli. His finding that "the proportion of cells with Feulgen negative nucleoli" (*i.e.*, plasmosomes) "increases at the expense of other cells" (*i.e.*, those with 'heterochromatic nucleoli' which are probably chromocenters) may be interpreted as showing that chromocenters, like plasmonucleoli, are subject to changes in neoplastic tissues.

The overall picture for the above data is that an increase in nucleolar size occurs with great frequency in neoplastic cells.

7. Much additional evidence on the heterochromatin-nucleolar relationship is at hand (7, 20, 54) and will be treated more in detail in a subsequent paper.

In summation, in addition to known differences according to tissue and developmental stage, the nucleolus varies in number and size under influence of nutrition, trauma, malignancy, diurnal rhythm, alterations in the mitotic cycle and differences in the amount of nuclear heterochromatin.

In its responsiveness to a wide variety of stimuli it is reminiscent of the chromocenters. Actually all this evidence is indicative of a simple and obvious fact, that the chromosomes, with their heterochromatic contacts with the external environment, the chromocenters, and the nucleoli to which the chromocenters are linked, directly as in the SAT-chromosomes, or indirectly through the anastomoses of the resting stage (or, to recognize the interpretation of the coiling school, through the approximation of many fine, extended minor gyres), constitute a sensitive reaction system whose parts would be expected to show changes correlated with changes in the environment.

Most of the experimental work in the past has been on isolated parts of this system. What is needed is an extensive and careful investigation of the correlated changes under controlled environmental conditions. And such studies can not properly be made without detailed knowledge by the experimenter of the morphology of the cells with which he deals.

THE NUCLEOLUS IN ALLIUM

In *Allium* there are generally two nucleoli of equal or subequal size which may fuse into one. These show corresponding positions along the equatorial axis of the sister cells, and are associated with the two SAT-chromosomes whose trabants are borne on short arms (31, 82). Ordinarily they would appear in the proximal region of the nucleus, but because of the strong anaphase and telophase contraction undergone by chromosomes of this species, the regular position with respect to the longitudinal axis of the cell is very much obscured. Sometimes the nucleoli are proximal, sometimes in the middle of the cell, more rarely to the distal pole. Heitz points out that because of the frequency with which this species is used in studies of mitosis, the regular equivalence of position of the nucleolus on the latitudinal axis, which is so conspicuous in other forms, remained long undetected.

Intranucleolar inclusions have received extensive but withal desultory mention in the literature. They occur as vacuoles, refractile crystals and chromatic granules. From investigation of a wide range of preparations of different types, in the collections of several universities, I can confirm in part Montgomery's observation that "vacuoles are normal structures in nucleoli, since they may be seen after the most diverse types of fixation and their size and number are not only limited for the particular cell, but are also different at different periods in the metamorphosis of the nucleus . . . young nucleoli are homogeneous and vacuoles first arise when they have increased in size" (66). I am not sure that the size and number are limited for the type of nucleus, however. Considerable variation seems to exist.

Other than the increase with age, the manifest diversity shows no sequence. Sometimes several vacuoles occur in a single nucleus; sometimes one large vacuole is present; at other times a highly refractile crystal; or again, several of these. Sometimes such a crystal may be seen surrounded by a light aureole, as though enclosed in a vacuole. This enclosing vacuole may be even more pronounced, as in the cases where the crystalline body was found in active Brownian movement. Figure 15 is of a nucleolus of one of the large xylem cells in Dr. Rafalco's Feulgen preparations imbedded in clarite. The refractile body in this case was enclosed in a quite definite vacuole of lighter substance, and exhibited a constant nervous zig-zag movement within its boundaries. Inspection at the time of writing shows the motion still continuing, although the preparation is now two years old.

In the older literature nucleolar granules and crystals have been designated as "nucleolini". Montgomery's paper (66) has a good bibliography on this subject. He cites Lavdowsky as having found a small granule in a central vauole in the nucleolus which he took for a centrosome in the process of origin; and also Van Bambeke's description of the nucleolinus in *Amaurobius* as "doue d'un mouvement très vif". "This interesting phenomenon", comments Montgomery, "certainly deserves investigation though it is not impossible that the supposed nucleolinus was in reality a micro-organism enclosed in the vacuole of the nucleolus". The picture in *Allium,* however, shows clearly that we are dealing with a body which, if an artifact, at least occurs with considerable frequency in many types of preparation.

"In agreement with the majority of observers", states Montgomery, "I can attach no particular morphological significance to the nucleolinus; it appears to be only a detached portion of the nucleolar ground substance, to be in most cases absent, and when present, to vary greatly with regard to size, position and number . . . many structures . . . described as nucleolini are in reality minute vacuoles which from their refrangibility appear to be granules".

Nothing vital has been added to our knowledge of this body since Montgomery's time. Carleton (6) devoted a special paper to its presence in mammalian cells, but his conclusions concerning its autonomous division seem questionable. If one examines the wide range of appearances assumed by the nucleolus in *Allium* the common denominator seems to be the existence of three separable components, a darker matrix, the lighter, more refrangible vacuolar substance, and the highly refrangible crystalline bodies. The last could of course be mere condensations of the second. Carleton comments that the impossibility of seeing nucleolini *intra-vitam* may be due to their having the same refractive index as the nucleolar substance; moreover, the fact that the Cajal technique in his forms shows them to possess an affinity for silver not shared by the other nucleolar substances, seems to indicate their chemical distinctness. Hughes-Schrader recently described the living nuclei in the iceryine spermatid as "homogeneous spheres in each of which lies a nucleolus with one or more minute, attached or embedded, refractile bodies" (38).

In conclusion, the nucleolus has long been known to consist of two or more components, differentiable by a wide variety of fixation techniques. For future research, isolation and analysis of these elements seems indicated, rather than further attempts to determine the contents of the nucleolus as a whole. A step in this direction has just recently been taken (79). The nucleolar inclusions in *Helix* oocytes were found on chemical analysis to have a lower amount of basic and non-basic proteins, and to show a more intense phosphorus test and more acidic reaction than the nucleolar fluid.

The authors interpret this as signifying a greater concentration within the inclusions of nucleotides and simple polypeptides. The rich phosphorus and nucleotide content of these bodies in *Helix* suggests the possibility that their movement in *Allium* may somehow be a manifestation of their own kinetic energy.

EUCHROMOCENTRIC NUCLEI

I turn now to the resting stage of the prochromosomal or euchromocentric type of nucleus, so carefully and extensively investigated by Doutreligne. The strong resemblance of this to the resting stage of the endosome type of nucleus in the Protista will be seen by a glance at her Figure 1 (15). The prochromosomes lie on the periphery of the nucleus, and a large plasmosome, very darkly staining in iron haematoxylin, occupies the central region. The nucleoplasm is typically homogeneous without a trace of any network.

The prochromosomes are always slightly elongate and rest against the nuclear membrane throughout their length. Sometimes they are dumbbell-shaped through a constriction in the middle which, as shown by its subsequent history, is the region of the centromere. The number of euchromocenters is in the neighborhood of, and never exceeds, the diploid number of the species. That this configuration is characteristic of euchromocentric nuclei, in general, was established by Doutreligne after examination of a number of forms.

The plasmosome shows a division into a clear central region and a darker peripheral region. In aged cells with large prochromosomes the nucleolar mass is much reduced; Doutreligne suggests there may be an inverse volumetric relation between the two.

In concluding the discussion of the resting stage, a possible distinction should be noted between the true resting or metabolic stage and the interphase, intermediary or interkinetic nucleus. The second type occurs in meristematic tissue where divisions are rapid; the first is found in the root cap and in the zone of elongation where division ceases and differentiation is pronounced. In the first type the nucleus is usually round and reticulation complete. The second type runs all degrees from a state where the nucleus remains in an ovoid or kidney-shaped telophase condition, and the chromosomes are distinct as they pass into prophase, to the typical rotundness and reticulation of the true resting nucleus. These differences must be taken into account in considering the various pictures of early prophase.

Early prophase in general. The onset of prophase is marked by four important parallel events.

First, an apparent increase of chromatic substance in the region of the chromocenters, as though they were acting as centers of condensation. These bodies are most conspicuous at each pole; as the process continues, the iron haematoxylin technique shows aggregation of chromatin occurring along irregular zig-zag lines running between these granules. This process is not unique in *Allium*. The literature is replete with examples where prophase increase in chromaticity begins in the vicinity of the nuclear membrane.

Second, the achromatic matrix in which the chromocenters and intranuclear granules are imbedded becomes visible in lateral view as bands of darker substrate alternating with narrower and lighter bands of nucleoplasm (Fig. 8). Polar sections show the arms of these emerging chromosomes as more or less equidistant in the uniform hyaline cytoplasm.

Third, the nucleoli begin to diminish in size, and in haematoxylin preparations their staining capacity decreases as that of the chromosomes increases; Feulgen preparations show diminution in size but no change in color or color intensity.

The fourth important event, rotation of the nucleus, is one which may not always take place but does so with great frequency. It was noted by Heitz (33) (see especially his figures 1, 2 and 8*b* for *Vicia,* designated by arrows). He gave no reason for its occurrence, however. This rotation of the nucleus resembles animal telokinesis and often begins in both daughter cells as early as telophase. Figure 17 represents diagrammatically the relations in early prophase of two daughter nuclei in which such rotation has already begun.

In the past this motion has perhaps been concealed by the frequently asynchronous division cycle of daughter cells and also by the fact that nuclear rotation is three dimensional and therefore frequently obscured in sectioned material. The occurrence of such rotation is also suggested by the number of cells found oriented, as in Figure 8. The rarity of longitudinally or diagonally oriented metaphase plates rules out the possibility that these very frequent pictures are those of cells about to undergo lateral division in the root tip. This rotation eventually brings the nucleus around almost to the point of reversing its polarity, *i.e.,* through 180 degrees. Formation of the metaphase plate completes the reversal of telophase polarity. Bělăr (3) saw the connection in *Tradescantia* between this shift and metaphase plate formation, though his interpretation is slightly different from mine, as is noted in detail below.

Prophase nuclei are thus to be distinguished from late telophase by several marked characteristics (compare Fig. 8 with Fig. 3). As Koerperich (40) pointed out for the forms she investigated, the spaces between the chromosomes as they emerge are at first much narrower than the actual chromosome bands; as prophase proceeds the spaces become wider, the chromosomes more undulant. The limits of the achromatic matrix are not so clearly defined as in telophase, due to the longitudinal attenuation of the chromosome elements as a whole. Sharp (80) states that the prophase nucleus is "of larger size and more evenly rounded contour" and distinguishable "by the absence of a similar sister nucleus in the same or adjacent section". I frequently find cases where the sister nuclei are in a similar stage of the mitotic cycle, although the tendency is for the development to be asynchronous.

Since the present paper is concerned with intranuclear movements and the coiling cycle, we will consider these events in detail for the early prophase period. As noted before, some cells undergo no resting stage, but pass into prophase in their telophase pattern, the chromosomes separated by clear interstitial spaces, their arms rounded out against the sharply convex nuclear membrane. The metabolic nucleus, on the other hand, enlarges and becomes more rotund. The chromosomes reappear in prophase as more nearly parallel although undulant lines (Fig. 8). In both types some arms of the V's and J's lie against the nuclear membrane; other arms lie in the central region of the nucleus. All, however, are in contact at their centromere apices with the nuclear membrane. This association of the centromere with the nuclear membrane is clearly shown by Levan for *Allium ursinum* (see his figure 10*a* (46)). Thus the prophase chromosomes re-emerge, preserving much of their telophase relationships to each other and to the nuclear membrane, despite the masking of this essential orientation by a certain amount of shifting in the nucleus and by the pronounced sinuosity of the chromonemata. Figure 8 is of an early prophase which shows favorably the polar and distal chromocenters, the wider spaces between the emerging chromosomes and the undulation which may be indicative of both a writhing motion on the part of the chromosomes and the initiation of those changes in position which are to continue through middle prophase.

With regard to the details of intra-chromosomal structure, three problems present themselves: (*a*) the number of the chromonemata, (*b*) the degree and manner of coiling, and (*c*) the exact relation of the threads to the chromosomal matrix.

The preceding telophase showed clearly each chromosome arm as consisting typically of two chromatids coiled around each other in the fashion of a double caduceus (Fig. 3). But the emerging coils of the early prophase appear single. Bonnevie (1) was able to find traces of early prophase duality for *Allium cepa* in very few cases. Koshy (42) believes that in the resting stage the duality is present but obscured by the full extension of the double coil, and states that "each chromosome has been observed to persist as two interlacing threads in all stages". His figures 22 to 24 show early prophase chromosomes as single. Other workers report the same rarity of clear cases of doubleness for other forms. Sharp (80) states that prophase chromosomes may appear single or double, depending upon fixation or other circumstances. As a matter of fact, the appearance of the chrômosome in Fig. 12, from a nucleus slightly less advanced than that in which the chromosome in Fig. 13 appeared, shows how labile the involved structures may be. The interconnecting and parallel strands of the former suggest doubleness, but nowhere is there any clear-cut evidence, and in the subsequent stage (Fig. 16) the chromonema appear unmistakeably single.

The sequence of Figs. 12, 13 and 16 illustrates how obscurely the coils arise or reappear. Bonnevie originally showed that safranin techniques reveal a more or less regular spiral, while the iron haematoxylin methods bring out zig-zag bands. These conclusions have been confirmed by most modern workers. But the very early prophase stages are confused in the safranin preparations, and for details we must rely on the haematoxylin methods.

Koerperich describes for the chromosomes of her forms an irregular chromatophile network disposed mostly on the periphery of the achromatic substrate. *Allium* presents a similar picture in the emergence of the chromonema from chromatophile lines connecting the granules which are for the most part distributed on the periphery of the achromatic bands. Here one does not see a spiral or even a continuous zig-zag (Fig. 12), but rather the arrangement described by Koerperich—crosspieces often marked by chromatic thickenings, these sometimes arranged in sinuous lines running from one border to another of the chromosomes. "Il serait vain de vouloir décrire avec precision tous les aspectes qu'on observe sur un même chromosome. . . . Ces aspectes sont extrement divers" she concludes.

The common picture from all these observations is the predominantly peripheral distribution of the chromatic substance at the interface between the matrix and the nucleoplasm. Where spirals are seen, most of the gyres are applied to the chromosomal boundaries. Where zig-zags appear, the nodes of the bands are usually located at these boundaries and marked by chromatin accumulations. Where a network is described, as in Koerperich's paper, the chromatophile granules are depicted as lying mainly at the outer chromosomal limits.

Early prophase in euchromocentric nuclei. The above data apply to the reticulate type of nucleus. For nuclei of the euchromocentric type Doutreligne describes an increase at the onset of early prophase, in the size of the nucleus and in the size and chromaticity of the euchromocenters. These show clearly the constriction of the centromere region. No change in the nuclear cavity occurs at this time. The nucleolus remains large and in the center of the nucleus as in interphase.

Middle prophase in general. Middle prophase in *Allium* may be defined as the period when the chromosomes lengthen, thicken, again show the prophase split and undergo the maximum amount of movement in the nucleus. At this stage many of the chromosomes show a tendency to apply both arms to the membrane, while retaining the centromere attachment. It is likely that in this period the shifts and changes take place which account for the differences in metaphase plate configurations from one cell generation to another. With the lengthening of the chromosomes the gyres diminish in number (Fig. 16). Although Koshy describes the split, which now appears, as restoring the diamond-shaped appearance of the two intertwined chromosomes seen in telophase, his Figs. 26 and 26*b* resemble more closely the chromosomes of my Fig. 2. I would also be more in agreement with Koerperich's Figs. 40–44 for this stage, which show the sister chromatids clearly parallel for considerable distances or interrupted by the bends of what may be considered relic coils. The cleft between the chromatids becomes more and more visible, the number of coils or bends diminishing until the stage is reached which is represented in my Fig. 4. Explanation of the coiling cycle calls for a tightening of the coils in each individual chromonema accompanied by the simultaneous untwisting of the sister chromonemata. Under this interpretation the chromomere-like bulges on the sister chromonemata seen in Fig. 4 may be the coils produced in the former process.

I am in agreement with Koshy (42) that during this period the arms of the chromosomes become progressively and more closely applied against the nuclear membrane until the entire nucleus eventually becomes more or less hollow, the nucleolus lying in the central region, leaving the nuclear cavity otherwise clear, except for some arms which touch the nucleolus. The apices of the V-shaped chromosomes lie for the most part very flat against the nuclear membrane, so that from a polar view the chromosomes appear like a cone of hairpins flattened against the inner surface of a hollow sphere (Fig. 9).

It should be remarked of middle prophase that viewed from any other than a direct lateral view, the orientation of the chromosomes to the division pole is obscured, and what one sees is a confused mass of chromosomes winding about in the nucleoplasm. I find, however, that such apparently random distribution never occurs at any time in the whole mitotic cycle, and that chromosomes remain roughly polarized and in contact at their centromere ends with the nuclear membrane, even though considerable shifting about may take place as the chromosome arms which filled the nuclear space come to lodge themselves more and more against the membrane.

Middle prophase in euchromocentric nuclei. I arbitrarily designate as middle prophase for Doutreligne's euchromocentric type of mitosis that stage in which the nucleus enlarges and the euchromocenters lengthen from their point of contact with the membrane inward, *i.e.*, into the cavity of the nucleolus. As the lengthening proceeds, the increase in chromatin is always preceded by the emergence into view of a forerunning achromatic portion of the chromosome. This sometimes appears clearly double (Doutreligne's

Figs. 3 and 4). The process continues throughout prophase, the chromaticity of the chromosomes increasing in length on both sides of the achromatic constriction, always spatially preceded by an achromatic region less sharply contrasted with the colorless nuclear sap. The achromatic regions are Feulgen-negative; chromatic regions, Feulgen-positive. Doutreligne reports no evidence of any spiral structure. The chromosome is seen to be in close contact with the nuclear membrane, not only in the spindle attachment region but a short ways along the chromatic regions to either side of this (her Figs. 4 and 5).

Late prophase in general. During late prophase, in favorable preparations, each chromosome may be seen surrounded by a smooth, hyaline matrix, delimiting it from the nucleoplasm. Traces of these sheaths or vesicles may be noted as early as middle prophase. By late prophase they become very clear. They are something quite distinct from the diffraction halo which appears around opaque bodies in a transparent medium. The problem of their nature will not be considered in this paper. I am, however, convinced of their reality.

Koshy describes for *Allium cepa* a conventional spiralization cycle in which the sister chromatids unwind progressively through prophase. I was unable to establish this as an exact and unvarying sequence, since very often the sister strands of middle prophase seem parallel through a great part of their length. This condition is seen, although rather obscurely, in Fig. 2. Most late prophase chromosomes, however, are as in Fig. 6 which corresponds to Koshy's Figs. 26 and 26*b* (42) and Koerperich's Figs. 48 and 50. And from this point on I can substantiate both authors in reporting a progressive unwinding of the sister chromatids accompanied by a condensation and increase in their chromaticity due to the coiling up of the chromonemata which presumably occurs in each sister chromatid. The centromere, although often discernible as an achromatic bridge in middle prophase, was not again distinguishable until very near the termination of this process, which occurs on the metaphase plate (Figs. 5 and 7).

Even late prophase stages may have been described as a spireme (see especially Fig. 13 (74)). Certain fixatives will apparently cause a confluence of chromosome ends, thus forming a serpentine chain, where other fixatives reveal discrete, polarized chromosomes as my Fig. 2.

Late prophase in euchromocentric nuclei. Late prophase in the euchromocentric type of nucleus is marked by steady advance of the chromatic portion of the chromosome, preceded by emergence of the forerunning achromatic portion, until eventually the chromosome is chromatic throughout its length. The chromosomes are now about twice the length they will finally have in metaphase, and many of their number are in contact with the nucleolus (Doutreligne's Figs. 6 and 143). Chromaticity has, as it were, been drawn distad over the chromosome like a glove. The bearing this has on the theoretical nature of heterochromatin will be noted in a subsequent article. No spiralization was apparent to Doutreligne, and the doubleness reported in early prophase is no longer in evidence. The chromosomes now begin to contract in preparation for metaphase. The nucleolus remains unchanged.

Prometaphase in general. The rapid transition which occurs between late prophase and metaphase is characterized by three events. First, the 90 degree rotation must be carried a little further to about 135 degrees, since in the greater number of observed cases the nucleus, just before dissolution of the membrane, is tilted at an angle similar to that shown in Fig. 17. It seems likely that this is in some way equivalent to the telokinetic movement of animal nuclei, and that the nucleus is to swing around in an arc of 180 degrees when the chromosomes go on the metaphase plate.

The second event is the dissolution of the nuclear membrane, which generally takes place while the nucleus is in a tilted position. This paper has purposely ignored all cytoplasmic phenomena, but here we must note the appearance, beginning back in middle prophase, of the two conical hyaline polar caps, encompassing the nucleus in the manner shown diagrammatically in Fig. 9. The chromosomes continue in contact with the nuclear membrane at the centromere region and at the extreme tips of both arms—at the centromeres and telomeres, to employ a convenient terminology. The chromosomal sheaths or vesicles are quite clear in many preparations and persist even after dissolution of the nuclear membrane.

The picture at the dissolution of the nuclear membrane is one of an influx of cytoplasmic material and an intermingling of cytoplasm and karyolymph and the substance of the dissolved nucleus in the regions between the vesicles. Flemming preparations show the intervesicular substance as cloudy, like the surrounding cytoplasm, not hyaline as was the old nucleoplasm. I have seen the same situation in Mann-Kolatchev preparations of grasshopper spermatocytes where the sharp boundary between osmiophilic cytoplasm and osmiophobic nucleus is changed at metaphase to areas of osmiophilic substance intercalating the large osmiophobic tetrad chromosomes.

The third event is the projection of the centromere regions of the chromosomes onto the equatorial plane. This may occur somewhat in the diagrammatic way shown in Fig. 9. For the formation of a typical metaphase with apices concentrically directed and arms radiating outward to the poles, the plane of the polar circle indicated by the inner ellipse of dotted lines must shift until it coincides with the equatorial plane. As the centromeres move toward the equator, some chromosomes come to lie with both arms directed to the same pole, and some, as would be the case with chromosome A, Fig. 9, with one arm directed to each pole.

The ends of the chromosome arms do not, however, leave their peripheral position, but continue to describe in space the outlines of the old nucleus which may be thought of as having rotated through 180 degrees from its old telophase position. The equatorial plate thus formed is oriented at right angles to the long axis of the root-tip, so that subsequent anaphase division results in an increase in root-length rather than in root-width.

Bělăr (3) described the post-telophase rotation of the nuclei and the independence of this motion in the two sister cells. By selecting the two terminal "Staubfadenhaarzellen" in *Tradescantia,* he obtained a fixed point of reference, and found that in this particular case only one cell actually rotated, namely, the one destined to divide again. The other remained more nearly in its old position. In the onion-root tip all the cells in a division field are presumably in a mitotic cycle, and both daughter cells would be expected to undergo movement.

Bělăr attributed this nuclear shift to the effect of cytoplasmic streaming. Yet in order to account for an identical rotation in *Tradescantia* root tip tissue where the apparent proximity of the nucleus rendered propulsion by cytoplasmic streaming improbable, he postulated that the prophase stretching of the chromosomes themselves turned the nucleus. I have seen something of this nature in the narrow xylem cells of the onion-root tip. Here the chromosomes have ample room to lengthen along the long axis of the cell, but, nevertheless, in middle prophase they migrate to a laterally directed position approximately 90 degrees from the telophase pole, inside a nucleus which undergoes little movement or change of shape. They appear, as it were, to crawl around in the narrow space in achieving a new polarity. The change of position in this case seems independent of plasma currents, or a stretching of the chromosomes, or any movement to secure "Lebensraum". More plausibly, it is a response on the part of the chromosomes themselves to whatever factors, internal or external, are producing the division figure.

Bělăr explains the formation of the metaphase plate in a manner approximating mine. But the larger number of prometaphase transitions I have examined in the onion-root tip show a nucleus slightly tilted, as though swinging over to a complete 180 degree rotation. This tilting seems best to explain the situation in which some chromosomes have both arms to one pole, some have both arms to the other, and still others, with one arm to one pole and the other oppositely directed. A glance at chromosome A, Fig. 11, will show how in the movements depicted this chromosome might come to lie in the last mentioned position.

According to Bělăr, metaphase configuration is the result of two factors, one of movement of the centromeres to the equatorial plate, the other the guidance of the intercalary spindle elements which tend to direct the chromosome arms vertically. He mentions, however, that it is the arms in the center which best show this alignment. Outlying arms do not seem subject to this discipline. I believe that because Bělăr was at this point working with living cells, he overlooked the rôle of the nuclear membrane. His views may be incorporated with those of the present paper by stating that the metaphase pattern is the result of four factors: (*a*) the movement of the centromeres to the equatorial plate, (*b*) the former relations of the telomeres to the nuclear membrane, (*c*) the vertical nature of the spindle substance which intercalates the chromosomes, (*d*) a possible continuation of the mutual repulsions which maintain equidistance of the chromosomes within the nucleus.

Before leaving this subject I should remark that late prophases which show the pole directed along the axis of the root do occasionally occur in the onion-root tip. These may be due to one of three situations: (*a*) no nuclear rotation has taken place, (*b*) rotation has occurred through 180 degrees prior to the dissolution of the membrane, and (*c*) a laterally dividing cell has undergone the usual 90 degree rotation from its original spindle pole. There is no way of determining which is the case without observation of living cells in a state of division.

As noted, the nucleoli usually disappear with the nuclear membrane. With Flemming fixation the chromosomal vesicles or sheaths remain intact. Bouin and Gilson fixations tend to produce a certain amount of distortion, but even here their presence is detectable. Thus the chromatic material does not really lie free in the spindle substance, as is classically depicted. Viewed at one plane the interface between a vesicle and the spindle substance may simulate a fiber. On focussing up and down, however, the "fiber" may be seen to follow the rounded contour of the vesicle cylinder. Possibly some of the "fibers" described by the older cytologists were in reality such vesicle boundaries. In any event, their presence appears to contribute to the characteristic striations of the spindle, along with the traction fibers proper which may be seen extending from the kinetochores.

The metaphase chromosomes have now achieved their maximum chromaticity and characteristic shortened J and V shapes. The centromere may again emerge as an achromatic region (Fig. 7) or as a chromatic projection (Fig. 5). The uncoiling of the sister chromatids finally brings the spiralization cycle for the mother chromosome to an end, producing two parallel chromatids in which the anaphase split for the next division is already beginning, although it is not always visible (Fig. 10). The slight waviness of the two daughter chromosomes in this figure may indicate the new double coils. Two chromosomes of the diploid sixteen bear satellites, separated from the main body by a constriction.

Prometaphase through metaphase in euchromocentric nuclei. Doutreligne did not concern herself with intranuclear movements of the chromosomes. Her account of prometaphase is thus probably incomplete. She defines this stage as the one following the maximum growth of the nucleus and the maximum length of the chromosomes, when the nucleus begins to diminish in size and the chromosomes to contract. Definitive metaphase chromosomes are short rods, one half the maximum prophase length, with a submedian or intermedial centromere constriction. No explanation is given of how the chromosomes attain their metaphase position, which is very characteristic of this type of nucleus. In her illustrations they appear lined up on the equatorial plane, coinciding with it throughout their length, each daughter half so exactly superimposed upon the other that from the polar view they appear as one chromosome. All chromosomes are completely contained within the keg-shaped spindle body. This is in clear contrast to *Allium* where at metaphase the sister chromatids are still slightly twisted with relation to one another, and the arms of the chromosomes more or less describe the outlines of the vanished nuclear membrane.

Anaphase. As noted, the nucleoli usually disappear with the membrane. Separation of sister chromatids may begin at any point along the length of the chromosome, as a part of the general uncoiling process, but true anaphase movement to the poles is of course initiated in the spindle-fiber attachment region. The poleward movement is classic and regular in V's and J's, depending upon its location.

The chromosomes are now more slender, but still smooth in contour and densely stained. The satellites are clearly visible. It will be recalled that in the spiralization cycle the individual daughter chromonemata are presumably becoming more tightly coiled as the sister chromatids uncoil from one another. The former coiling up process is supposed to account for the packed, densely staining appearance of the metaphase-anaphase chromosomes. Since the separation of the sister chromatids now terminates the coiling cycle of the mother chromosome, we may expect a similar uncoiling process to being at anaphase in the individual daughters. Fig. 11 is of an anaphase chromosome whose outlines strongly suggest the familiar double caducean figure which would result if this were the case. In Feulgen preparations the intertwining of the daughter chromonemata is even more clearly visible. A double caducean figure for both chromatids of *Allium* chromosomes, *i.e.*, a quadripartite as far back as metaphase, is represented by Mensinkai (61).

Telophase in general. As the chromosomes converge toward the poles, they seem to contract and form a dense chromatic lump, obscuring the individual members. This is the classical "tassement polaire" which many authors claim to be an artifact. In Feulgen preparations the chromosomes are very closely approximated—the clumping clearly occurs, although the outlines of the individual chromosomes are more readily detected. Even in some iron haematoxylin preparations the chromosomes are distinct, though contiguous. Relativity in degree of clumping and varying adsorption phenomena may account for these differences. About this time a new cell wall forms at a point midway between the poles.

Late telophase reorganization of the nucleus involves several concurrent processes. The chromosomes lengthen, diminish in chromaticity, and loose their smooth outline. In preparations other than Feulgen the nuclear membrane and nucleoli simultaneously reappear. As the chromosomes continue to fade out they retain marked chromaticity in two regions. These are particularly conspicuous in haematoxylin preparations. At one pole the centromere regions remain chromatic as the more distal or euchromatic region of the chromosome fades. The same is true at the opposite pole where the extreme distal ends of the chromosome touch the membrane. Thus the polar and distal chromocenters, whose incrementation signalized the onset of prophase, now re-emerge in prophase together with the nucleoli.

Feulgen preparations do not show these as distinct, large granules, but merely as more deeply staining regions of undefined extent. Their more definite appearance in iron haematoxylin preparations may be due to adsorption phenomena, to the greater density in these regions or to the presence of plastin elements, to list by implication the major interpretations concerning the nature of the haematoxylin stain.

Safranin and Feulgen preparations clearly show the emerging chromonemata as coils, and the former show them enclosed in a chromosomal matrix of different staining than the interstitial nucleoplasm. Fig. 3 also shows this condition very well. In most such preparations, however, the caduceus appears as a chain of diamond-shaped apertures (Fig. 18). Either of these configurations may have been derived from the loosening up and decreased chromaticity of an anaphase chromosome, as is pictured in Fig. 11. The apparent distribution of the chromatin to the periphery of the chromosomal matrix is confirmed in cross-sections of reorganization nuclei which show the chromosomes as consisting of a lighter core enclosed in an irregular chromatic cortex, the former considerably darker than the surrounding nucleoplasm. The occasional appearance of single chromonemata raises the question, why do the threads which are double in telophase emerge as single in the succeeding prophase? Feulgen preparations typically show double coils, but here and there appear strands that are single.

As late telophase passes into resting stage, the nucleoli continue to reverse their prophase behavior by growing larger, smoother and more pycnotic. The chromosomes continue to lose their staining capacity. Constellations of smaller granules appear in addition to the polar and distal chromocenters. These are of all gradations of size, but the larger ones are found mostly in the region of the nuclear membrane. A finer analysis of chromosome transformation shows that coils, double spirals, or zig-zags lose their definiteness as the granules emerge (Fig. 14). Koerperich describes this stage in her forms as a chromatin network first disposed chiefly on the surface of the achromatic substrate. Thin chromatophile bridges traverse the substrate, but the preponderance of the chromatic substance is at the periphery. To judge from what is seen when the nucleus is examined as a whole, in *Allium* those chromosomes whose outer boundary apparently constitutes the nuclear membrane show on the inner surface of this boundary scattered granules of larger than average size.

Increase in the complexity of the reticulum takes place by the appearance of numerous smaller granules and connecting chromatin bridges and by continued growth of the cell, changing it from an ellipsoid or ovoid to a distinctly globular form. This extends the individual chromosome arcs and gives them a more parallel alignment than in the bulging, tangerine-like pattern of the telophase. It is quite possible that the establishment of chromatophile bridges between the granules and gyres of neighboring chromosomes, as has been described by Sharp (80) and many others for plant mitosis, may also contribute to this continuous increment in the reticulum. All that can be said is that the growing network seems to obliterate the intercalary nucleoplasmic space. Neither can anything be postulated at present concerning the fate of the achromatic chromosomal substrate, *i.e.,* whether it flows along the chromatic network adhering to it as a correspondingly fine, attenuated and consequently indetectable sheath, or whether some of its substance enters into the nucleolus, or whether, again, it dissolves into the nucleoplasm.

The end result of this process is reconstitution of the resting nucleus and completion of the mitotic cycle. Polar, lateral and distal chromocenters now appear with a firmament of smaller granules of varying size, in which lie the two nucleoli, now at their maximum in smoothness of contour and staining capacity.

Telophase in euchromocentric nuclei. Reconstitution of the interphase or metabolic nucleus with its large, centrally located, endosome-like nucleolus is of course different from the reconstitution of the reticulate nucleus with its relatively small nucleolus or nucleoli. All the chromosomes seem to participate in nucleolus formation by the emission of a substance from the terminal regions, whereas in the reticulate nucleus the nucleoli typically reappear in the same location, on the same chromosome with which they were associated in prophase. Again, during this process, the chromosomes of the euchromocentric nucleus fade entirely from view, except for the euchromocenters which are applied against the nuclear membrane at the division pole. The reconstituted nucleolus is at first located in contact with or close to this pole—later, as the nucleus becomes spherical, it assumes the central position characterizing the resting stage. Doutreligne considers that her investigations establish the identity of the euchromocenters as centromere regions which maintain their metaphase condition through interphase, and that the whole body of the chromosome really maintains its identity at this stage, although it disappears from view, since it is otherwise difficult to explain the distad procession of the achromatic filament which precedes the distad increase in chromaticity as the chromosome comes into view.

DISCUSSION AND SUMMARY

My main purpose in this critique has been to describe mitosis in a familiar laboratory and pedagogic form, the root tip of *Allium cepa,* as a continuous process and as an integrated whole; to this end the spiralization cycle has been traced parallel with the movements of the chromosome within the nucleus. Secondly, I have been interested in bringing down to the elementary level of instruction a number of important fundamentals which, while known to cytologists, have not yet been incorporated in our elementary textbooks and laboratory models and manuals. To both these ends I have been at some pains to assemble the established facts of cell structure and division in *Allium* and to fill in one or two existing gaps. Still a third purpose distinguishes the present paper. A great deal of the speculation and discussion concerning the significance and function of cell organs has been conducted at the purely genetic or biochemical level, without consideration of pertinent data on their topography and distribution in the cell. This is particularly evident in the current literature on the nature of heterochromatin, as will be developed below. I propose, therefore, a reconsideration of this question in terms of the allocation of heterochromatin in the cell, which will be the subject of my next paper.

The facts herein brought out, taken into consideration with many others in the cytological literature, all emphasize the importance of the rôle played by membranes and interfaces in mitosis, *viz.*:

(1) The widespread phenomenon of karyomery, both normal and abnormal, would seem to indicate two things: (*a*) that the ability to form a small nucleus with its own membrane and often with its own nucleolus is a generalized property of chromosomes; (*b*) that, as Conklin has suggested (9), "the resting nucleus is not a single structure; it is composed of units each of which has the properties of an entire nucleus".

(2) Of the three morphologically distinct regions of the chromosome, the centromeres, the telomeres and the secondary constriction, the first consistently and the second frequently, are points of contact with the nuclear membrane bounding the external cytoplasmic environment. The third is a point of contact with, or region of formation for, a distinct intranuclear body, the nucleolus.

(3) Of the two morphologically distinguishable types of chromatin, hetero- and euchromatin, the former is typically associated with precisely these two regions, the nuclear membrane and the nucleolar surface. In *Allium* the polar granules appear at or near the juncture of the centromere region with the nuclear membrane. In the euchromocentric nuclei, the euchromocenters remain throughout the resting stage as two heterochromatic areas on either side of the centromere, applied against the nuclear membrane. In *Allium* the distal granules are points of contact of the chromosomes with the nuclear membrane ((33) and my Fig. 8); the lateral granules are similar points of contact between the chromonemata and the

membrane at various points between the poles. The nucleolus organizing regions in *Zea* and the trabantochromocenters of the resting stage in *Allium* and other forms are associated with or lie near the nucleolar surface. We shall return to these points in discussing the nature of heterochromatin.

(4) The chromosomes are in association with the nuclear membrane throughout the mitotic cycle until at or shortly before formation of the metaphase plate. They do not, as often erroneously depicted, lie free in the karyolymph. In late telophase and early prophase the centromere connections can still clearly be observed, and the evidence from (3) above indicates that they maintain this connection throughout interphase. As prophase progresses, the chromosomes undergo various shifting movements leading to their even more extensive lengthwise application against the nuclear periphery, the apical regions and the terminal regions of the arms becoming very closely applied.

The pattern of the apices on the equatorial plate resulting from the nuclear movements shown in Fig. 9 is thus a rough mercatorial projection of the global polarization of late prophase, while the distal ends, even after considerable shiftings of position, continue to describe in space the sphere of the old nuclear membrane with which they were in contact.

Now, although the contact of the centromere regions with the nuclear membrane in *Allium* can readily be established by the position of the proximal chromocenters for the entire period of interphase and early prophase in which they remain distinguishable, in later prophase this distinction is lost; we lack the critical evidence which would be at hand were we able to trace individual chromosomes through a complete cycle. But the great frequency with which such contact or proximity can be clearly demonstrated, particularly in the loop or hollow ball stage (Figs. 2 and 9) is very convincing. Evidence from other forms, moreover, supplies strongest confirmation of the constance of this association.

In Doutreligne's study of the euchromatic nucleus there can be no doubt of the constancy of an intimate contact, for all the chromosomes of the genome from telophase through prometaphase. In these forms the primary constriction remains visible with its heterochromatic blocks on either side, closely applied to the nuclear membrane until its metaphase dissolution, and a chromosome count is at least approximately possible at all times. The constancy of the association is thus established in these forms, and there seems to be no good reason why the reticulate type of nucleus should be any different, particularly in view of Levan's findings that in *Allium* the heterochromatic blocks are mainly at the centromere region ((51*a*) taken into consideration with my Figs. 1 and 8).

I have been able to follow several marked chromosomes in the genial cysts of the grasshopper *Podisma alpina.* Here the chromosomes are telomitic and undergo no interphase dissolution. In this form the constancy of the association of the heterochromatic proximal end with the nuclear membrane or with the interface of an enclosing chromosome vesicle is unmistakable (unpublished observation).

In *Drosophila* the salivary gland centromeres are all located in the large compound chromocenter, which in turn is located at or very near the nuclear membrane. These chromocenters, or at least the alpha chromatin portions, are similarly located in the smaller, non-glandular somatic cells. In highest probability we are dealing with the same constancy of kinetochore alignment near the nuclear periphery.

A most elegant demonstration of the constancy of such association with the nuclear membrane is to be found in the remarkable intranuclear division cycle of *Barbulanympha* and related forms, in Cleveland's classical volume on *Cryptocercus* and its Protozoan symbionts (7*a*). Here the chromosomes maintain contact with the nuclear membrane by what seems to be the homologue of a centromere region. These contacts are often marked by indentations in the nucleus; and their movement follows the somewhat complicated centrosome history throughout the mitotic cycle.

Even the tiny chromosomes of fungi may exhibit the same arrangement. McClintock (59*a*) reports for *Neurospora* that the two main heterochromatic segments are located adjacent to the centromere.

In five separate cases which have been studied, then, in five separate groups, the higher plants, the Orthoptera, the Diptera, the Protozoa and the Fungi, the kinetochore or its probable homologue exhibits a regularized association with the nuclear membrane.

Such association is deducible from evidence in other forms. It is certainly implicit in the finding of Ribbands (71*a*) that nonhomologous centromere-telomere associations occur at the nuclear membrane in the meiotic prophase of *Habropogon.*

(5) From their topography and physiology we might consider the heterochromatic centromere, telomere and nucleolar chromocenters as liaison regions. I pointed out above that direct sensitivity of chromocenters to environmental stimuli, because of their peripheral location, is a more plausible explanation than that offered by Darlington and La Cour for the undercharging of these regions in metaphase under action of low temperature—namely, nucleic acid starvation and a competition for nucleic acid between inert and active genes. There are other speculations which can be reviewed in the light of centromere and telomere associations with the nuclear membrane. It has been suggested (14*a*) that "concentration of the heterochromatin near the centromeres must for some reason be advantageous", since "inversions in natural populations rarely involve breaks in proximal sections". But heterochromatin probably occurs here for the same reason that cities occur near the mouths of rivers. The region is a junction. And if the centromeres were attached or contiguous to the nuclear membrane, then inversions would be rare in this vicinity for mechanical reasons. Because the heterochromatin of the centric region in *Allium* retains its stain longer than other kinds, it has been concluded (53) that the prochromosomic heterochromatin is of a special kind, necessary for the function of the centromeres. Such heterochromatin may or may not be necessary to centromere function. A much simpler explanation is at hand if the nature of the centromeres as liaison regions with the cytoplasm is considered.

(6) Euchromatin appears as a series of tracks between these heterochromatic regions of nucleolus and nuclear membrane. In the resting stage the chromocenters are ranged on the periphery of the nucleus, and a reticulum, or mass of closely approximate gyres, fills the nucleus; the nucleoli are known definitely to be attached to this reticulum or mass of gyres, and in *Allium* the points of contact are heterochromatic, as are the chromocenters. As the definitive chromosomes emerge in prophase, this arrangement is not lost. The chromosome arms apply themselves against the nuclear membrane, and against the nucleolus in its vicinity, as though they were performing some function as lines of communication between the nuclear and nucleolar surfaces. Again, both chromocenters and nucleoli have been shown to be sensitive to the same stimuli, *viz.*, trauma, temperature, malignancy, *etc.* The combined morphologi-

cal and physiological evidence seems to indicate that chromocenters, chromosomes and nucleoli are in some manner a reaction system; and that the various forms which chromatin assumes, and the changes which it undergoes in the mitotic cycle, are a shifting series of pathways along which transfers and transformations of energy and substance take place between chromosomes and karyolymph and between nucleus and cytoplasm. Further evidence for this hypothesis will be presented in the sequel to this paper.

(7) Structural polarity in the cell is distinguishable in an antero-posterior gradient and in a surface-interior gradient. The first is reflected in chromosome polarity. The larger chromocenters are located at the proximal, or active division, pole, the smaller chromocenters at the distal, presumably less active, pole (Figs. 1, 8). Greater concentrations of chromatin exist at the surface of the nucleus than in the interior, and the same is true of the individual chromosomes. Chromatin is usually found concentrated more toward the surface of the chromosome matrix than in the interior (Figs. 12, 14, 18). The suggestion that these gradients in the concentration of chromatin may be somehow tied up with surface-interior and antero-posterior oxidation-reduction gradients is a tempting one. Morphological polarity of the cell would then in some way be connected with its energy metabolism. My succeeding paper will enlarge upon the evidence for this possibility.

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FASCIATION

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INTRODUCTION

Because of its widespread occurrence among vascular plants, fasciation has become a relatively well-known plant monstrosity, or teratological abnormality. Morphologically, fasciation involves a change in vascular plants from the normal round or polygonal stem, or axis, to one which, as a whole or in part, becomes flat, banded, or ribbon-shaped. While the change in the main axis of the plant is often the most striking feature involved in fasciation, all parts of a plant have been recorded as altered through this phenomenon (53, 55, 113, 136, 157, 197).

When fasciation occurs in a stem, the early seedling growth stages are cylindrical or normal. As the plant approaches maturity, the growing point becomes broader and broader (53, 55, 136, 197), often attaining a width of six to eight inches and sometimes several feet, as exemplified in the so-called "cancer" forms of the giant cactus of Arizona (*Carnegia gigantea*) (83, 198) and the hoop pines (*Araucaria cunninghamii*) of Australia (6, 197, 198). The growth region ceases to be a normal cone-shaped point, becoming linear and comb-like in some cases, or developing numerous growing points in others. In the former case, due to inequalities in growth, the affected stem may terminate in a coiled, snail-like helix, ram's horn, or shepherd's crook, or it may become distorted into a most intricate and grotesque tangle of coils and partly-broken stem (53, 55, 113, 197, 199). In cases in which the growth region develops numerous growing points, clusters of branches produce a witch's broom effect (53, 55, 90, 136, 199). Occasionally in certain plant species, such as the Mummy or Crown type of garden pea, the main axis when fasciated becomes not only abnormally enlarged, but also hollow, forming a ring fasciation (17, 31, 55, 133, 150, 174, 197). Generally speaking, in herbaceous plants, *e.g.*, asparagus, the main stem alone is affected, the branches remaining normal; but in *Lobelia syphilitica, Oxalis crenata* (89), *Celosia cristata* (the cockscomb) and *Oenothera* (113) several stems may become fasciated. When fasciation affects the inflorescence as well as the stem, it progressively increases the number of parts, so that the gynoecium in the most irregular flowers represents the most extreme departure from the normal condition, as determined by the increase in number of locules (197).

However, the most strikingly significant characteristics of fasciation are the increase by weight and volume of tissue over that of the normal type of the same variety or species and the relative loss of control over the growth area, resulting in irregular and unpredictable growth aspects. In some respects, this places fasciation in the category of galls; in other ways it has analogies to cancer (99, 199).

Aside from its purely scientific interest, fasciation has economic values. Cristates among succulent plant families, *e.g.*, Cactaceae, Euphorbiaceae, Crassulaceae, are much-prized, often high-priced, collector's items (203). The common cockscomb, *Celosia cristata*, and its numerous plumose forms, is one of the world's most common garden flowers (78, 111, 191). The commercial value of the tomato owes its existence largely to the increase in fruit size brought about by a prehistoric fasciation mutation in some tropical American valley, together with an appreciation of the value of this change by the bronze-skinned cultivators (128, 130, 162, 192, 208, 209). Possibly maize or Indian corn belongs in the same category (30, 38, 194). Some of the very large-fruited types of strawberries, if well grown, have a high percentage of fasciated fruits (42, 209). The best varieties of ocas, *Oxalis crenata*, of the Andean Indians are said to be those with fasciated tubers (89). One of the most interesting—although certainly not the most beautiful—suites of furniture I ever saw, owned by a Mexican general, was made from leguminous wood covered with Wooden Roses, or Roses of Hell, a fasciation which results from parasitism by a species of mistletoe (*Phorodendron* sp.) (58), *Psittacanthus calyculatus*, according to F. L. Wellman.

OCCURRENCE AND DISTRIBUTION

Except for *Fucus,* which has sometimes been mentioned as having a type of fasciation, I have been unable to find records of fasciation in the non-vascular groups of plants. More or less typical fasciations have been recorded from at least 107 of the 303 families into which Engler divides the living vascular plants.

Although only about one-third of the vascular families are represented in Table 1 as having had fasciated individuals, this does not mean that such phenomena do not occur in most or all of the other families. Records are very scattered in the literature, and many of the families are small and may be restricted to little-known regions. From my studies as well as from investigations of the literature, there seems to be no basis for supposing that the individuals of any particular vascular family may always be exempt from fasciation. It is more reasonable to adopt the view (90) that under the "proper" conditions, all vascular plants may become fasciated. DeVries believes this character is entirely absent from some plant families and that it is an old latent character from an evolutionary standpoint, which, under favorable environmental conditions, reappears from time to time. It is said to be more common in dicotyledons than in monocotyledons (53, 55). Seedlings and herbaceous plants with spear-like growing points are said rarely to be fasciated, since they are liable to less injury from friction in pushing themselves through a relatively hard surface.

Many investigators (53, 55, 90, 197, 198) have noted that fasciations are more numerous in certain genera and species than in others. Aside from hereditary forms, as exemplified in certain races of *Celosia, Pisum, Nicotiana, Solanum, Oenothera* and other genera, this phenomenon is extremely common in the Compositae, Cactaceae, Ranunculaceae, Meliaceae, Liliaceae, Simarubaceae, Euphorbiaceae and Crassulaceae. *Erigeron, Asparagus, Delphinium, Cichorium, Euphorbia, Lilium, Mattkiola, Melia, Ailanthus, Echium, Taraxacum, Sedum, Echeveria, Cereus, Opuntia, Lactuca* and *Rosa* are genera which frequently produce magnificent examples of fasciation, often in quantities of two or three dozen at the same time in the same general area (197).

Examples of this anomaly have been recorded from trees, shrubs, vines and herbaceous plants. In trees and shrubs, branches and

TABLE 1

PLANT FAMILIES IN WHICH FASCIATED INDIVIDUALS HAVE BEEN RECORDED

Acanthaceae
Aceraceae
Aizoaceae
Amarantaceae
Amaryllidaceae
Anacardiaceae
Anonaceae
Apocynaceae
Aquifoliaceae
Araceae
Araliaceae
Asclepiadaceae
Berberidaceae
Betulaceae
Bignoniaceae
Boraginaceae
Bromeliaceae
Cactaceae
Campanulaceae
Capparidaceae
Caprifoliaceae
Caryophyllaceae
Casuarinaceae
Celastraceae
Chenopodiaceae
Clethraceae
Combretaceae
Compositae
Convolvulaceae
Cornaceae
Crassulaceae
Cruciferae
Cucurbitaceae
Cyatheaceae
Cycadaceae
Dioscoreaceae
Dipsacaceae
Droseraceae
Epacridaceae
Equisetaceae
Ericaceae
Euphorbiaceae
Fagaceae
Gentianaceae
Geraniaceae
Gesneriaceae
Goodeniaceae
Gramineae
Guttiferae
Haemodoraceae
Halorrhagaceae
Iridaceae
Juglandaceae
Labiatae
Lauraceae
Leguminosae
Liliaceae
Linaceae
Loranthaceae
Lycopodiaceae
Lythraceae
Malvaceae
Meliaceae
Moraceae
Musaceae
Myrtaceae
Oenotheraceae
Oleaceae
Orchidaceae
Orobanchaceae
Oxalidaceae
Palmae
Papaveraceae
Phytolaccaceae
Pinaceae
Piperaceae
Plantaginaceae
Plumbaginaceae
Polemoniaceae
Polygonaceae
Polypodiaceae
Primulaceae
Punicaceae
Ranunculaceae
Resedaceae
Rhamnaceae
Rosaceae
Rubiaceae
Rutaceae
Salicaceae
Sapindaceae
Sapotaceae
Saxifragaceae
Scrophulariaceae
Simarubaceae
Solanaceae
Sterculiaceae
Tamaricaceae
Taxaceae
Thymelaeaceae
Tiliaceae
Ulmaceae
Umbelliferae
Valerianaceae
Verbenaceae
Violaceae
Vitaceae

"water sprouts" are most frequently altered, while among vines and herbaceous plants, the main stem is usually affected. On maples and plums I have collected fasciated branches two or three feet in length, while cut-back trees of *Ailanthus* and china-berry (*Melia azedarach*) have provided some striking specimens of water-sprout fasciations four to five feet long. One year severely pruned crepe-myrtle bushes (*Lagerstroemia indica*) produced several examples, but I have looked in vain for them on the much-pruned privet (*Ligustrum*) hedges. The giant cactus of Arizona (*Carnegia gigantea*) apparently quite commonly produces magnificent cockscomb fasciations, judging from those pictured on postcards and in various articles on southwestern North American deserts.

The most bizarre fasciation I have ever seen described and illustrated is that in an Araucarian hoop pine, *Araucaria cunninghamii,* in a park in Beecroft, Australia. Twenty years ago the fasciated region had affected 26 feet of the top of the main stem, resembling in form a giant *Celosia* cockscomb.

Among vines the best examples known to me are in the common Chinese wisteria (*Wisteria sinensis*), the pumpkin (*Cucurbita* *pepo*) (107, 186), the tropical American monkey ladder (*Bauhinia splendens*) (160) and the Chinese yam or cinnamon vine (*Dioscorea batatas*) (19).

The fasciated pumpkin vine (186) grew in a well-manured garden of a rich cotton merchant in Coimbatore, South India. The main stem was normal and three-quarters of an inch wide for 15 feet, from which point it gradually began to increase in width for about 20 feet, ending in a much-furrowed, hood-shaped, striated structure about a foot broad. The fasciated portion bore leaves, tendrils and a few small male flowers which withered and dropped off in the bud stage. Huge crowds of people came with offerings to worship it, believing it to be an incarnation of Nagasarpa or King Cobra. Since the merchant was pious and charitable, it was believed that Nagasarpa had appeared in his garden in the form of a pumpkin vine, indicating that the god Vishnu was pleased with this man and had sent his blessing through his messenger, the Nagasarpa.

The monkey ladder is a common jungle vine in tropical America, often ascending over 100 feet to the tops of the tallest trees. I have collected it in Bolivia, but have never seen its flowers or fruits, since these generally occur at the tops of tall trees. I have never grown it from seed, but would welcome the opportunity, since it appears to be a fasciation that has become the normal condition, successfully adapted to a wide range of natural conditions. Very little seems to be known about it, and until its growth habits have been looked into more carefully, its classification as a fasciation must remain in doubt.

The fasciated cinnamon vine (19) grows in a garden in Brantford, Ontario, Canada. Although the stem always dies from frost, the tuber has for several years sent up a new stem which at first is normal for several feet, then becomes typically fasciated. In 1946 the flattening began at about 20 feet.

Among herbaceous plants, annuals, biennials and perennials seem to be equally affected, although the most striking examples probably occur in biennials. Particularly broad ribbon forms having stems two to three inches in diameter are not rare in Viper's bugloss (*Echium vulgare*), common snapdragon (*Antirrhinum majus*) (61, 62, 103) and the weedy evening primroses (*Oenothera biennis* (193) in particular. Annuals sometimes have a high percentage of fasciated population in a given area. This has been recorded for canning peas (90% fasciated) (17) and for certain chenopodiums and erigerons (90, 197). Among perennials fasciations in larkspur (*Delphinium*) (73), buttercup (*Ranunculus*) (197) and dandelion (*Taraxacum*) (22, 108, 135, 136) are well known. In *Ranunculus cymbalaria* (199) a culture of a few square feet frequently contained a dozen or more fasciated flowering stalks. In a similar area under favorable conditions dandelions have produced 35 or 40 fasciated flowering heads. Fasciations are very common in hybrid delphiniums.

Halophytic and hydrophytic environments are unfavorable to the production of fasciations, as I can find no records of such plants in the species characteristically growing under these conditions. For over 30 years I have collected fasciations and looked for them without success in plants characteristic of such conditions. Nor have my numerous correspondents sent me any, although I am indebted to them for many examples from other groups.

Among xerophytes fasciated individuals are very numerous. Anyone looking through a catalogue of succulent plants will be impressed by the number of kinds of cristates, particularly in the cactus, euphorbia and stone-crop families. Xerophytic surroundings do not inhibit the creation of fasciations, as is shown by the frequency of their appearance in cactus hedges (90) of the dry Mexican plateau, as well as in other succulents of desert regions. Furthermore, in species in which fasciation is hereditary, artificial drouth conditions, while lessening the degree of expression, never completely inhibit it so far as *Celosia cristata* and fasciated tobaccos (197) are concerned. *Sedum* is characteristically a xerophytic genus, but a well-known fasciated variety of *Sedum reflexum* has been known for two centuries. *Echeveria whiteii* (11, 199), sent by me from Bolivia to the Brooklyn Botanic Garden in a non-fasciated form, after ten years spontaneously produced fasciated stems which were difficult to propagate and were finally lost.

Among mesophytes fasciation probably attains its greatest diversity and most frequent expression.

Geographically fasciation is known from practically every region of the globe.

COMPARISON OF THE FREQUENCY OF FASCIATION IN WILD AND CULTIVATED PLANTS

Fasciation occurs as commonly in wild plants as in those cultivated, judging from a survey of the literature and from my own observations. This appears to be the case in spite of the better opportunity for observing them in cultivated plants. This also fails to support a not uncommon belief among biologists that variations more frequently occur among domesticated life forms than among those in the wild.

Common in Holland are wild fasciated plants of *Crepis biennis, Aster tripolium, Geranium molle, Taraxacum officinale, Oenothera lamarckiana, Raphanus raphanistrum* and *Pedicularis palustris* (55). Fasciated *Erigeron canadensis* is in the United States very frequently found in weedy lots, along roadsides and in abandoned fields (90, 197). Fasciated individuals of *Rudbeckia hirta* occurred by the hundreds in a field near Hainesport, New Jersey, in 1893. *Ranunculus bulbosus* was similarly affected in a meadow near Haddonfield, New Jersey, in 1893 (197). *Lepachys columnaris* not infrequently presents this phenomenon in considerable numbers. Numerous occurrences of fasciation have been recorded for the common dandelion (22, 108, 133), in both Europe and the United States. Genera in which fasciations occur frequently in uncultivated areas are *Lactuca, Echium, Chenopodium, Opuntia* and *Eupatorium.*

As fasciation occurs in both an hereditary and a non-hereditary form, it is to be expected that cultivated plants will furnish more individual cases, since, in general, the hereditary form is more likely to be perpetuated than if it were subject to natural selection. Further, in cultivated plants it is more likely to be observed.

Among cultivated plants excellent examples of fasciation are produced by sugar beets (147, 183), maize (57, 91, 106), peas (31, 124, 138, 197, 199, 200), sweet potatoes (32, 199), pineapples (33, 82, 87), climbing roses (178, 197), lilies (1, 88), raspberries, blackberries, New Zealand spinach (*Tetragonia expansa*), sunflowers, sweet peas (23, 114, 115, 146), asparagus (136), tomatoes (128, 130, 192, 209), cockscombs (75, 78, 197) and strawberries (42). Canning peas (17) in fields in Maryland occurred one season in which not over 10% of the vines remained unfasciated. Conard (32) finds the sweet potato to be so commonly fasciated in all parts of the United States, where it is an important crop, that he concludes it to be an hereditary phenomenon. In Puerto Rico (33) the Smooth Cayenne pineapple has produced over 25% fasciated fruits. Other varieties of pineapple also produce fasciated fruits, but the percentage is not so high. Fasciated maize ears (57, 197) were frequent in cornfields 25 years ago, and strains breeding true to this character were isolated in both dent and pop corn varieties. Among strawberries (42, 182, 209) the Fairfax under certain conditions is notable for its fasciated fruits, but many other varieties also produce them, though less frequently. Probably all varieties of tomato (128, 192, 199, 209) other than the small-fruited, two-loculed types, such as cherry and currant, have fasciated fruits, flowers and, not infrequently, stems. The cockscomb (*Celosia cristata*) (55, 78, 111) and its plumose forms are hereditary fasciated types of *Celosia argentea.* The Crown or Mummy pea, cultivated in Europe for over 300 years, is a fasciated form of the garden pea (197, 200). Along a Boston parkway I once counted over 50 fine examples of stem fasciation in a planting of hybrid *Rosa wichuriana* (197), roughly 25% of the plants being fasciated.

CLASSIFICATION

Variation may be viewed from two angles: morphologically, which takes into consideration external form, color, anatomical structure and other physical features; or physiologically, which involves the conditions necessary to produce the character, its transmission from generation to generation through seed, its vegetative propagation, and the factors, hereditary or environmental, that affect its minimum and maximum development.

MORPHOLOGICAL VARIATIONS

Structures

Fasciations have been classified by various investigators from a morphological standpoint into linear, bifurcated, multi-radiate and ring or annular types (55). These categories are based on the stem. Linear is the most common form, in which the stem is flat or ribbon-shaped. Bifurcated refers to a division of the linear type into two flattened branches. In the third, exemplified in fasciated *Amaranthus speciosus,* the inflorescence or stem tip is separated into three or more short branches. Tri-radiate types are common in the Compositae. *Digitalis lutea* and *Celosia cristata* develop quadriradiate forms.

Ring fasciations are rare. In this type the main axis or stem becomes distended into a funnel-shaped structure with the inner cavity somewhat freely exposed to the atmosphere. They have been described in *Taraxacum officinale* (136), *Pisum sativum umbellatum* (Mummy pea) (131, 197), *Veronica longifolia, Peperomia maculosa* (55), *Nicotiana tabacum* (197) and various species of *Oenothera* (113). The distinctions between all these types are morphological and they may all be present in the same species or even on the same plant. Dissimilarities in anatomical structure may make them more typical of some species than of others.

A more detailed morphological classification based on the special plant structure that was altered involved six different types, but these have since been shown to occur within the same species and even on the same plant. However, in order to understand clearly the nature of the changes caused by fasciation, a somewhat detailed description of the effects produced in the various plant parts is necessary. Main stems, underground stems, branches, leaves, inflorescence, flowers, fruits, roots and seedlings will be considered.

Main stem. This is the plant structure most commonly altered and most frequently described as fasciated. It also shows the most striking effects, so that one can often spot it from a speeding automobile. In the garden pea, tobacco, and annuals in general, the main stem at first appears normal but gradually broadens until the characteristic ribbon or ring type is formed, and the apex changes from a point to a line or comb. Frequently the main stem alone is affected. In those perennials and biennials in which a rosette is formed the first year, the ribbon-like stem may arise from the base, *e.g., Oenothera, Saxifraga* and *Crepis.* This is not true of *Gilia rubra.* A fasciated main stem may or may not terminate in an inflorescence. It may become a mass of coiled and broken tissue, or it may develop many growing points, forming a mass of branches or witch's brooms.

Underground stems. Fasciations of these have been described in *Spiraea sorbifolia* (55), *Solanum tuberosum* (197) and oca, *Oxalis crenata* (89). In *Oxalis* the tubers transmit the character.

Branches. Branches in many fasciated plants remain unfasciated, for which there are various causes. In *Pisum* and *Nicotiana* (197) generally only the main stem is altered, while in *Oenothera* (113) and *Celosia* both may be affected and frequently are so. In shrubs, since there is no main stem, several branches may be changed. In trees and large shrubs a small branch or two may be fasciated.

Branches never produce so striking or so extreme expressions as those of the main axis.

Leaves. Leaves are usually modified by fasciation through their relation to the main axis. Changes in number and position are common; changes in size and shape relatively uncommon. Changes in number and position (phyllotaxy) are exceedingly irregular in character, as illustrated by a fasciated form of *Nicotiana* (197), in which the phyllotaxy was very irregular and the number of leaves fluctuated through an arc of 28 to 160, as compared with its normal form with regular phyllotaxy and 18 to 24 leaves, with a mode of 20. Occasionally these plants produced at the same stem point two leaves which, though apparently fused, may have grown from one primordium. A fasciated plant of *Hymenocallis senegambia* (34) produced all gradations between normal single leaves, semi-double leaves and two independent leaves at the same node. Leaves, if the number be greatly increased on a fasciated plant, may be smaller than those of normal stems. In plants pinnately leaved, *e.g.*, clover (100), fasciation may increase pinnae number.

Inflorescences. Inflorescences on fasciated plants either have the number of flower-bearing stems increased, or these regions may be suppressed altogether. In the former case the increased number of pedicels or peduncles produces a witch's broom effect, as occurs in fasciated forms of *Erigeron, Sonchus, Nicotiana, Lactuca, Lilium* and some plumose types of *Celosia cristata.* When the floral twigs are shortened and the main floral axis becomes a fan-shaped, truncate structure, a combed or cockscomb type is produced, as exemplified in the ordinary *Celosia cristata* and in staminate cones of *Phyllocladus glauca* (197). Between these two types of fasciated inflorescence all gradations exist. In either type bifurcate and multiradiate crowns may occur. Inflorescences have been observed on fasciated plants only slightly modified from normal, perhaps only in the "apparent" fusion of two or more of the terminal pedicels. In extreme cases of fasciation in tomatoes the central or terminal flowering stem possesses many sterile flowers. Sterile flowers also occur on some forms of *Celosia cristata.* Mulberry and pineapple inflorescences in fasciated individuals result in greatly increased number of fruit units, thus modifying the shape of the subsequent fruit.

Flowers. Flowers on fasciated stems are usually altered in structure, though this may not always be true. In the Mummy pea the flowers are normal, whereas in fasciated tobacco and tomatoes the flowers present striking modifications in repetition, linear arrangement, atrophy and hypertrophy of parts. Repetitions (polyphylly) may occur that involve only one or any combination of the four whorls of organs. The calyx is most likely to be modified, and the likelihood of alteration of the other three whorls follows in the order of their axial attachment. In fasciated forms of tobacco and tomato the gynoecium frequently presents the most extreme effect, as judged by repetition of parts and various other abnormalities. In *Geranium molle fasciatum* (55) repetition of flower parts takes place in an orderly manner as a series of duplication of whorls, the normal flower being five-lobed, while those on fasciated plants have 10, 15 or 20 lobes. In tobacco (197) and tomato (209) there was no such regularity. Flowers on a single abnormal plant of *Nicotiana* may vary from the normal 5–5–5–2 to various combinations of sepal, petal, stamen and ovary locule number. Sepals varied up to 12 per flower, petals up to 22, stamens up to 25, and ovary locules up to 21. Table 2 gives ten flowers representing the most and the least abnormal from a fasciated tobacco plant wtih 69 leaves.

TABLE 2

Flower	1	2	3	4	5	6	7	8	9	10
Sepals	7	6	6	6	7	8	8	7	7	6
Petals	12	9	6	11	13	6	8	7	12	7
Stamens	12	12	7	8	12	14	9	7	14	7
Ovary locules	3–3–3	3–3	4	4–3	6–3	8–5	5	3	4–6–4	3

Very frequently two or three pistils were present, as indicated in the table by 3–3–3, but the undivided ovary in such a case was 9-loculed. Pistils on these flowers were often incapable of functioning, owing to various forms of tissue proliferation in the stigmatic region. Styles were frequently shortened, coiled and united at their bases with an anther (staminody of the pistil). Stamens were altered in both filament and anther. Filaments were coiled, twisted, shortened and not infrequently abnormally united with the corolla. Anther deformities consisted of split anthers, anthers with small functionless pistils growing from them—in one case, two to three to an anther (pistillody of the anthers). Pollen or anther sacs varied from the normal four to six. Not uncommonly, anthers aborted all or part of their pollen before they matured (contabescence). Various cytological irregularities occurred, but none that resulted in the production of increased chromosome numbers in the progeny. Corolla and calyx were frequently split, especially in flowers nearest the point of greatest growth activity. Calyx and corolla lobes were often altered to an irregular shape and size. By a spiral twist the calyx and corolla occasionally merged. Two flowers were sometimes enclosed by the same calyx. In one instance flowers appeared consisting only of a corolla and a few stamens partly united on the side to a normal corolla. No doubling of corollas was ever found, but the flowers with high numbers of corolla lobes were greatly enlarged in circumference and often appeared to be as regular and symmetrical as normal flowers. In very few cases was there any striking effect on fertility. Only one plant in several hundred was completely sterile, and this appeared to be a mutation. There was some correlation, though not striking, between the degrees of alteration in different structures of the fasciated plants. The plants with the highest leaf numbers usually had the most abnormal flowers and often the broadest stems, but this did not necessarily follow. From Table 2, one can see that 6-lobed calyxes were combined sometimes with 3-loculed ovaries, and at other times with 7-loculed ovaries; while 8-lobed calyxes were combined with 5-loculed ovaries or with 13-loculed ovaries. Likewise, flowers with 7-lobed calyxes might have 3, 9 or 14 ovary locules. Of 850 flowers collected at random in approximately equal numbers from 34 fasciated tobacco plants, the modes for the four whorls were 7–7–7–4, the average being 7.03, 8.24, 8.48 and 4.86. Since the gynoecium is normally 2-loculed, while the other three whorls are 5-lobed, certain adjustments must be made in order to compare them. When these are made, the average alteration produced by fasciation in the flowers of one strain of the fasciated tobacco was calyx 0.80+, corolla 1.29+, stamens 1.39+, ovary locules 2.86+, demonstrating progressiveness of modification in the floral structures. The first flowers to bloom were much more abnormal as to form than the later ones, although both may have had similar numbers of flower parts.

In fasciated tomato plants (209), involving all the individuals of the large-fruited commercial varieties, similar conditions were found in the floral structures as those described for tobacco. In the most extreme case the ovary had 215 locules, the variety being Beefsteak. Locule number ranged in three other varieties from 3 to 18 in Break o'Day, 5 to 39 in Triple-L-Crop, 4 to 29 in Colossal, the normal condition being 2.

In *Glycine soja* (187), the soybean, some of the flowers on the fasciated form had two or more pistils and abnormal pods. *Antirrhinum majus* (61, 62), *Stachys sylvatica* and *Digitalis purpurea* produce modified flowers on fasciated individuals; while in *Phlox drummondii* (103), flowers on fasciated plants remain normal, as they do in fasciated peas.

Fruits. The seed capsules of fasciated tobacco plants were frequently distorted, hypertrophied and atrophied structures. Increased locule number corresponded with the increased number indicated under "Flowers". Fruits of a fasciated medlar (154) (*Mespilus germanica*) have been described twisted into ram's-horn shape, having 40 calyx teeth instead of the normal 5. In certain varieties of pineapple (33) the multiple fruits have from 2 to 71 crowns, the normal condition being 1. Fruits have been described with 3, 4, 5, 6, 7, 8 and 13 separate and compound crowns, or with fan-shaped compound fruits, enlarged by more or less continuous series of leafy crowns. The specimen with 71 crowns weighed 18 pounds, as against a normal 2 to 3 pounds. Some varieties are more commonly affected than others. Mulberries are another multiple-fruit type in which fasciated fruits frequently occur. Many varieties of strawberries have fasciated fruits, especially the earliest berries on some of the large-fruited forms. This is particularly true when these are grown under very favorable conditions. Some varieties (42, 209) are more subject to fruit fasciation than others. The Fairfax in North Carolina produces 30% fasciated fruits. Chesapeake produces more such berries than Blakemore. There appears to be an environmental condition that influences the production of such berries, even in varieties where it is a pronounced character. Clyde (32) is another strawberry variety frequently having large fasciated fruits. The writer once was given a box of cherries, most of which were double or fasciated.

Other than the pineapple, the tomato (120, 128, 162, 209) presents the most striking example of what the presence of this character can do in a fruit. All commercial tomatoes represent degrees of fasciation, as indicated by the increase in their locule number over the normal 2. Two-loculed fruits are characteristic only of the currant, cherry and other very small-fruited types. These represent the wild or normal condition. The abnormal condition begins with an average of three locules in Red Peach, and constantly increases in number in different varieties until in Triple-L-Crop, Brimmer, Colossal and Stone, among 30 varieties investigated, it reaches a beginning-of-the-season average of from 14 to 15 locules per fruit. The commercial varieties Marglobe, Rutgers and Pritchard average from 5.38 to 6.02 locules, while Oxheart and Beefsteak average 11.12 to 13.49. Some fruits with a very high locule number between 50 and 100 are twisted and gargoyled into fantastic shapes. As indicated under "Flowers", plants with such fruits may also bear a considerable number of fruits with relatively low locule number, depending somewhat on the weather and the length of time they have been bearing.

Occasionally, bananas (26) and squash fruits become fasciated.

Maize ears (57, 91, 197) are frequently fasciated. They were especially common in the old-fashioned cornfields of 30 or 40 years ago, and by inbreeding, races were founded on them. A rice popcorn variety known as "Bear's Paw" is not uncommonly grown. Reading in one of Luther Burbank's catalogues about a cross between Stowell's Evergreen Sweet Corn and Jerusalem corn, I sent for seed, which, when grown, turned out to be identical with Bear's Paw. Fasciated grains (106, 202) of maize have been described.

Coconuts (25) and walnuts are occasionally fasciated. A coconut has been described with 14 locules, all fertile, as contrasted with the normal 3, only 1 of which is fertile. Coconuts ranging from 4 to 10 locules have also been recorded.

Roots. Fan-shaped aerial roots have been found in the cactus (204), *Epiphyllum hookeri.* Striking examples of fasciation in orchid roots have been observed in *Saccolabium blumei* (34), *Aërides crispum* and *Phalaenopsis schilleriana* (5). Other examples occur in yams (*Dioscorea alata*). The root nodules on leguminous and cycad (80) plants are often typical fan-shaped fasciations.

Seedlings. Seedlings of dicotyledons not infrequently possess more than two cotyledons (55), and these may be more common in some fasciated plants than in others, although this is doubtful. In true-breeding fasciated races of tobacco, peas and cockscomb, I found that the majority of seedlings bore the normal cotyledon number.

Chromosome number. So far as the hereditary fasciated forms of peas and tobacco are concerned, no individuals showed any increase in chromosome number, although in tobacco (195, 197) there were numerous irregularities in meiosis. Dr. Hans Heyn (unpublished) investigated many types of fasciated peas in The Blandy Experimental Farm cultures and failed in all cases to find any but the normal numerical condition of seven pairs.

Histology. As was to be expected, a number of investigators found irregular increases in the number of vascular bundles. In an anatomical investigation of a fasciated rose branch (178), the following differences as contrasted with normal branches were noted: epidermis was thinner; cells were larger, quadrangular and disposed in a longitudinal series; stomata were more numerous and arranged in regular longitudinal rows; the cortical parenchyma was thinner; pericycle cells were larger in diameter and less woody; and the pith gave a much fainter tannin reaction. In fasciated flax (177), vascular bundle number was increased, but there occurred no change in shape or number of cells per bundle, although the fibers were coarser and more likely to be damaged by retting than normal stem fibers.

Theories

Two theories regarding the morphological nature of fasciated organs have been advanced. Moquin-Tandon (145) held that fasciation results from flattening or enlargement of a single growing plant. Linnaeus (122) believed that fasciation results from an increase in the number of growth points or buds, and that these, owing to crowded conditions, subsequently fuse. Masters (126) discusses the arguments for and against each theory and favors that of Linnaeus. Recent investigators (90, 149) of the anatomical structure of fasciations are inclined to agree with Moquin-Tandon. Studies of fasciation in the Mummy pea (31), in fasciated oenotheras (113) and in cockscombs and fasciated tobacco (197) lend no support at all to the Linnaean concrescence, or fusion, theory. According to the anatomical evidence from these investigations, fasciations result from enlargement of a single growing point.

Worsdell (204), however, favors a modified form of the fusion concept. He believes fasciation to result from a compromise between two inherent tendencies and rarely to be a case of real mechanical fusion in the Linnaean sense. Two opposed forces operate in the organism, one inducing integrity, the other producing plurality of parts. On this concept, fasciation in the higher plants is to be regarded as a reversion to the ancestral or primitive branching character of the lower plants, such as occurs in ferns, lycopodiums and algae. It is a congenital fusion of an unusual number of branches, fusing in one plane because of reversion to the ancestral condition. Reed (165), studying seedlings of various species of legume, supports the Linnaean concept.

PHYSIOLOGICAL VARIATIONS

General Considerations

From a physiological viewpoint, all variations may be placed in two categories, those inherited and those uninherited. Variations are discussed in terms of characters. Characters are rather indefinite entities, since their boundaries are capable of various interpretations. In genetics terminology, a character is not inherited, but results from the expression of one or more genes in a specific environment. In other words, a character (197) is the combined expression of a bit of protoplasm under a definite set of environmental conditions. Combinations of different genes with the same or different environments, and combinations of the same gene with different environments, may all result in what are termed the same character. Hence from this angle such a character as fasciation may result from many causes. Although the result will look the same and be designated by the same name, actually from a specific causal standpoint, such characters are very different.

Looking at the character fasciation from this viewpoint, it becomes relatively easy in many cases, by means of progeny tests, to distinguish between the inherited and the non-inherited form, even though the two types may occur in the same area in the same species at the same time. However, owing to our general ignorance of the extent of the various combinations of material and environment that will result in fasciation, the difficulties of classification are greatly increased. Environmental and hereditary effects are hard to disentangle.

As an example, DeVries (55) found that none of the races of fasciated plants that he investigated, when given the progeny test, bred true, although some of them almost did so. He also found that the percentage of fasciated individuals in such races could be increased by selection, but the permanency of the increase was always subject to environmental caprice. Even in a homozygous pure line, DeVries regarded the expression of this character to be inconstant, although all the individuals were presumed to have inherited it. He referred to such characters as "eversporting". In some of DeVries' cultures, over half of the individuals were normal, although the race was held to be a true-breeding fasciated race so far as transmission was concerned. Seeds of these normals, when selfed, gave progeny in which the percentage of fasciated individuals was nearly as high as that from seed of selfed fasciated plants. Some fasciated races were poor in regard to phenotypic expression, others were rich. In a poor race, in which few fasciated individuals occurred, selection was of but slight avail in raising the percentage. Such poor races were referred to technically as "half races". Rich races ("middle" races), of which *Crepis biennis* is a typical example, often gave as high as 50% or more fasciated progeny. I shall recount the history of this typical example in the Amsterdam garden as related by DeVries.

In Holland this species is frequently fasciated, and DeVries found two such plants growing among hundreds of normals in a meadow near Hilversum in 1886. From the normals he grew 100 plants in 1887–88, 12% of which were fasciated. He makes no mention of progeny tests of the two fasciated individuals. Seed from the 12% fasciated plants gave 120 plants, of which 40% were fasciated. From the 40% fasciated individuals, three of the finest were selected, and 30% of their progeny was fasciated. Continuing to grow progeny from the fasciated group in each generation, 24% were fasciated in the fifth; in the sixth, the percentage was high, though no figures are given. The seventh generation produced only 20% fasciates. The average percentage of fasciated individuals in each generation is said to be fairly constant, fluctuating between 30% and 40%. In 1895, 41 individuals grown from fasciated plants were abundantly fertilized with horn meal, which raised the phenotypic expression to 85% fasciates, the race under ordinary conditions at this time producing 20–40% fasciates. If these 41 plants had not been crowded, DeVries thought, every one of them might have been fasciated.

From this investigation DeVries' conclusions were as follows:

Races always consist of fasciates and atavists (normals). The proportion of fasciates varies greatly, often amounting to 40% or less, but not infrequently to more (*Geranium* and *Crepis* 65% and 85%; *Celosia cristata,* complete atavists very rare). Fasciated and normal individuals show transitional forms, though this is rare. The percentages of each are to a large extent dependent upon external conditions, since these conditions can transform atavists into fasciates, and *vice versa.* This transformation takes place during the plastic period in youth, before the character itself develops. Normals (atavists), as well as fasciates, produce fasciated offspring, the former often in proportions only slightly lower than the fasciates. Between fasciates and normals there is no fundamental genetic difference, in spite of the difference in their external forms. Fasciation is due to some internal hereditary quality, which, though often latent, becomes active in response to external conditions. Its wide distribution causes one to assume that it arose in some common ancestor of the forms which now possess it. Hence, phylogenetically, it must be very old. Both poor races and rich races may be strengthened or weakened in percentages of fasciated individuals by selection and treatment, but the limits between such races are never transgressed. Poor races cannot become rich races through selection. The external conditions being the same, the hereditary factor must be variable.

I have briefly summarized DeVries' studies of fasciation, since his investigations have been more extensive than those of any other worker. His conclusions represent an extremely complex interpretation of a set of facts that may be given a much simpler and clearer explanation in the light of more recent studies on fasciation.

Types

From a causal standpoint, I have arbitrarily classified fasciations into five groups for convenience in discussion. These are as follows:

1. Fasciations which, having been investigated sufficiently, according to recognized experimental procedures, breed true, and, in crosses with normals, obey orthodox genetic laws so that their genic bases may be ascertained.

2. Fasciations that arise due to various natural environmental causes, the selfed seed of which does not reproduce this character.

In genetics terminology they occur under various specialized environmental conditions and would be considered as non-inherited forms.

3. Fasciations that occur spontaneously, the initial cause of which is unknown, but which have been propagated vegetatively. Propagation through seed is unknown or imperfectly investigated.

4. Fasciations that are induced artificially by known procedures, which possibly in some cases have been asexually propagated.

5. Fasciations which have not been investigated experimentally and which remain unclassified as to their transmissibility.

Inherited Fasciations. True-breeding homozygous races of fasciated plants are now known in many species. Thousands of individuals of some of these races have been grown, under experimental conditions, without one normal appearing among them. This is true of *Pisum sativum umbellatum* (72, 138, 197, 200), the so-called Mummy pea; *Celosia cristata* (7, 55, 70, 71, 75, 78, 111, 169, 191), the cockscomb; *Nicotiana tabacum fasciata* (195, 199), the Dewey Sport; some races of *Zea Mays* (38, 57, 91, 126, 197), *e.g.,* Bear's Paw pop corn; *Phlox drummondii* (103), the annual phlox; *Pharbitis nil* (79, 93, 94, 205, 206), the Japanese morning glory; *Antirrhinum majus* (62, 103), the common snapdragon; *Capsella viguieri* (14, 15), a form of shepherd's purse; *Impatiens balsamina* (101), the common balsam; *Sesamum indicum* (153), sesame; *Glycine soja* (187), the soy bean; and most of the varieties of the cultivated tomato, *Lycopersicon esculentum* (128, 130, 131, 208, 209). An additional hereditary fasciated form of *Nicotiana tabacum* (27*a*) has been discovered, which first became known as a segregation product of an old established Canadian normal variety called "Belge", which was designated "Hundred-Leaf Belge".

So far as the history of these races is known, they all arose as mutations, in most cases appearing first in experimental cultures as recessive segregates, differing from the normal form in one or more genes. In *Antirrhinum majus, Phlox drummondii* and *Zea Mays* fasciate mutants appear to have arisen as in *Nicotiana,* more than once from relatively unrelated normal varieties.

The Mummy pea also has a long history. It is figured and described by Gerarde in 1597 under the name of "Scottish" or "Tufted Pease". Mendel used it as the source of one of the 14 characters in his classic seven pairs, first demonstrating fasciation as a recessive in F_1 in crosses with normal axillary flowered varieties. Such F_1's showed a ratio of 3 normals : 1 fasciate in F_2. This cross has been repeated many times, with the same results. I have made this cross, using at least 100 normal varieties, representing distinct types from all over the pea-growing world, including wild forms from Asia Minor and the peculiar varieties cultivated in China, Persia and Abyssinia. In all cases normal was dominant, and there was simple monohybrid segregation. Some crosses produced fasciated segregates that expressed this character to a less degree, but there never was any difficulty in distinguishing them from normals.

The Dewey Sport, *Nicotiana tabacum fasciata* (195, 197), first became known from a single plant found in Cuba in a field of the Cuban variety of tobacco. This single plant had a flattened stem, 152 leaves, abnormal flowers, and produced very little seed. For various reasons, discussed elsewhere, it was believed to have originated as a zygotic mutation, but in the light of present-day concepts regarding mutations, chromosomes and genes, this now seems improbable. If the original mutation had been present in the Cuban tobacco field in more than one individual, then one could consider that it was a gametic mutation and that the F_1 had been overlooked. If this were true the original mutant known as the "Dewey Sport" was an F_2 segregate. A possibility exists that Cuban conditions are not so favorable to seedlings of the Dewey Sport type as the conditions under which I grew them at the Bussey Institution in Boston. On such an hypothesis only one fasciated segregate chanced to survive. Due to the cleistogamous nature of *N. tabacum* it is believed that the Cuban strain in which the mutant arose was probably a natural homozygous pure line. According to all available accounts, the mutant, when selfed, bred true. Several lines were grown, derived from the least abnormal and the most abnormal individuals. These showed some degree of difference as regards abnormal expression based on leaf and flower structure counts, indicating that selection was slightly effective. My own attempts to modify the fasciated character in the two lines given me were inconclusive and were interpreted as ineffective.

These lines, when subject to crowding in pot cultures and other unfavorable growth conditions, continued to show the typical fasciated expressions, but in a less striking manner, since the plants were much reduced in size. No normals appeared. The two fasciated lines were crossed reciprocally with the normal Cuban variety in which the original mutant appeared. The F_1 was intermediate, and the F_2 generation was composed of typical fasciates, intermediates of the F_1 type, and the normals in the ratio of 1 : 2 : 1. F_3 plants, selfed, gave the results expected, the fasciates and normals breeding true, and the intermediates again producing a 1 : 2 : 1 ratio. No difficulty was experienced in distinguishing the three classes. So far as one could determine, no segregation of other characters took place in this cross.

Three other normal varieties and four species of tobacco were crossed with the Dewey Sport. The three normal varieties have been described elsewhere in great detail. The point I wish to emphasize here is that they differed markedly in flower, leaf and general habit characters among themselves and from the normal Cuban variety. The F_1's resulting from these three crosses were intermediate but differed in various degrees from each other and from the Dewey Sport × Cuban cross.

The F_3 generation of these three crosses presented a very different picture from the Dewey Sport–Cuban cross. Instead of an easily-classified, clear-cut 1 : 2 : 1 ratio, it was impossible accurately to separate the three classes without further breeding tests. In attempting to classify them by stem characters, such results as 1 fasciate to 5 normal, 1 fasciate to 9 normal, and 1 fasciate to 8.5 normal were obtained, instead of 1 fasciate : 2 slightly fasciate : 1 normal, as was recorded in the Dewey Sport–Cuban cross. Leaf count and counts of flower parts presented the same difficulties in attempting to distinguish between normals, fasciates and the intermediate F_1-like segregates. The ratio of abnormal to normal plants in these three crosses was very confusing; as in all other cases there was an excess of "somatic" normals, and this divergence from the theoretical expectancy increased as the apparent character differences between the two grandparents became more numerous. This excess of normals above that expected ranged from 2.8 and 4.8 to 9.7%. Many other characters were segregating. In the last cross there were scarcely any two plants alike among the 477 F_2 progeny.

From studies on subsequent generations grown from selfed F_2 individuals from these crosses, modifying factors were believed to be responsible for the difficulties encountered. Lethals are not considered as being involved. Segregates were discovered that bred true to the normal condition, which were believed to carry the gene for fasciation and which, in appropriate crosses, would express it.

The four species involved in the Dewey Sport × normal crosses were *N. glutinosa, N. paniculata, N. sylvestris* and *N. bigelovii.* The strain of the last-named species used bred true to a 3-loculed ovary, the other floral structures ranging between 5 and 7, 6 being very common. As was to be expected, the most abnormal of these F_1 species crosses was *N. bigelovii* × the Dewey Sport, followed by Dewey Sport × *N. sylvestris* and Dewey Sport × Cuban normal. The least abnormal F_1's were those involving the three normal *N. tabacum varieties.*

The second fasciation mutant in *N. tabacum,* previously referred to as "Hundred-Leaf Belge", also differed from its parent by one gene difference, but in crosses with another tobacco variety it gave a dihybrid ratio in F_2 (*27a*).

In maize numerous races of various complexities in regard to the inheritance of fasciation have been found and investigated (126). This character expresses itself strikingly in the ears. It appears to occur most frequently in the dent and pop corn groups. I have never found it among flour, sweet or flint varieties. Cutler (38) describes it in ears and tassels of a variety of Bolivian sweet corn, and it is said to be of not uncommon occurrence in pod or tunicate maize, where it is expressed also in both tassels and ears. Tassels of maize are extremely variable in regard to the branching habit. I have grown plants with a single unbranched spike. Ramosa maize may be a modified form of fasciation, since its much-branched ear recalls the witch's broom expression of fasciation in some tobacco crosses and in the plumose condition of some types of *Celosia cristata.*

The normal maize ear has been discussed as a type of fasciation (30, 38, 193); and to one who has seen types of pod corn with a simple, curved, slender, stem-like rachis instead of the usual cob, this possibility makes a very reasonable appeal. Particularly is this true when one sees how the fasciated character can be altered by modifying genes.

One group of investigators (55, 91) considers fasciation in maize to belong to the eversporting category of mutants, while another group (57, 197) finds true-breeding races in which the character, although always present, frequently fluctuates in expression. Experimental results indicate that fasciation may behave either as a dominant, a partial dominant or a recessive, depending on the varieties involved. In most cases the segregation is monohybrid. In one case the possibility of a dihybrid segregation was suggested.

The fluctuating character of its expression has been attributed by most investigators to environment, since ears on the same plant have varied from broad to but-slightly-flattened tips. Modifying genes have also been considered as playing a part in this fluctuation. From a family of pop corn heterozygous for fasciation, Emerson (57) selected four fasciated ears, the progeny characteristics of which are given in Table 3.

TABLE 3

Ear number	Number of plants grown	Character of progeny ears
1	18	All strongly fasciated
2	46	All strongly fasciated
3	15	12 more or less fasciated: 3 perfectly normal
4	28	Strongly abnormal, normal and all intergrades, making accurate classification impossible

Fasciation in the Japanese morning glory, *Pharbitis nil,* is conditioned by three recessive factors, one of which is either closely linked with the pear leaf factor or is to be regarded as another expression of this factor. Mono-, di- and tri-hybrid segregation occurs in F_2. Normals appear in fasciated strains which are interpreted as reverse mutations. Fasciations also occur, apparently indistinguishable from the hereditary types, which result from environmental causes and which produce only normal progeny.

Fasciation in sesame involves two pairs of genes, non-accumulative in type. Either normal allele is dominant. Flowers, as well as stems, appear to be altered in this plant through fasciation.

In *Impatiens* (101) both hereditary and environmental types have been described. The presence of both types in the same species has been recorded also for *Pisum* (17, 197), *Pharbitis* (94) and *Oenothera* (199).

In the soy bean (187), annual phlox (103), common snapdragon and *Capsella viguieri* (15) fasciation is recessive and gives a monohybrid ratio in F_2.

In the common chicory (185), *Cichorium intybus,* fasciation is very variable, although strongly inherited, but intermediate in expression in F_1.

Fasciation in the tomato undoubtedly is responsible for the high place it holds in the modern dietary world. Without the fasciation gene, the round, smooth, little 2-gm. red berry would still be an ornamental love apple. Normal fruits and flowers in this species are found in such small-fruited types as the cherry and currant tomatoes, and these may be, or at least more nearly represent, the wild type. Increased size (128) came about in prehistoric America through mutation and selection. According to some investigators (208), size and fasciation are intimately associated. Others (192) ascribe size to one set of complementary genes and fasciation to another such set. When cherry or currant types are crossed with modern cultivated varieties, the F_1 is intermediate in regard to fruit and flower fasciation. There seems to be considerable confusion as to segregation and gene identity in the F_2. From studies (209) now in progress at The Blandy Experimental Farm, it is hoped that further light will be thrown on the inheritance of fasciation in this plant.

Fasciations from various natural environmental causes, which are not inherited through seed. Although the literature teems with descriptions of fasciation in various plants and its inheritance, in most of the experiments no mention is made of the methods employed in studying it. This is particularly true of DeVries' experiments in which he grew many generations from fasciated plants belonging to different genera and species. Unprotected seed was apparently collected generation after generation, and from these he grew his eversporting rich and poor races of fasciated plants. Some of them may have represented heterozygous fasciated varieties, involving one or more pairs of genes. Others, so far as the data can be interpreted, may have been normal races, upon which, since his experimental cultures were grown in the same plots year after year, the environmental influence was reimposed on few or many individuals in each generation.

Investigations that do come under this head have been carried out on various *Oenothera* (113) species and on *Erigeron canadensis* (90, 197). In both groups fasciated plants under properly controlled conditions produced no fasciated progeny ascribable to inheritance. Microscopic injuries inflicted by the moth *Mompha* on the initial meristem of the growing point in *Oenothera* during the earliest growth stages were regarded as the primary cause. The greatest number of fasciations were produced under optimum conditions, showing an abrupt increase of nutriment to be the real cause, though this increase was made possible through alteration of the metabolism of the plant by the insect. Fasciations in *Erigeron canadensis* (90) have been ascribed to injuries caused by a Dipteran fly, *Cecidomyia erigeroni.* Progeny from abnormal individuals were all normals.

Spontaneous fasciations of unknown origin propagated asexually, but, so far as known, not through seed. In this class belong the numerous cristate forms found among succulent plants. Nursery catalogues specializing in the Cactaceae, Euphorbiaceae, Crassulaceae and Portulacaceae quote these for sale at a premium. Asexually propagated cristate forms of *Sedum, Echeveria, Opuntia, Neomammillaria, Cryptomeria, Cereus, Echinopsis, Adromischus, Lobivia, Neolloydia, Pachycereus, Salisia, Lophophora* and *Aeonium* are among those frequently listed. The cristate form of *Opuntia serpentina* is especially common and is sold under the name "Boxing Glove cactus". *Euphorbia lactea* in its crested form is also well known in collections. Small plants of these cristate or crested forms are often found in ten-cent store collections of succulents. According to the propagators of these oddities, many of them are successfully multiplied only by grafting them on normal forms, this being particularly true of the cacti. In some cases this is done primarily to induce strong growth. In some species the cristates or fasciates apparently revert to the normal form. That has been my experience with a fasciated form of *Opuntia* and with *Euphorbia lactea* var. *cristata.* So far as can be determined, no one has attempted to find out whether these cristates reproduce this character through seed. Although some of them produce flowers and fruit, there seems to be a question as to whether they ever produce viable seed (203). Because of the prevalence, under wild conditions, of crested individuals in a limited area, it has been maintained that these must have come from seed of an original mutant. Such plants could easily have arisen from one of the numerous environmental causes, as presumably happened to the field of canning peas in Maryland, or as apparently occurs frequently with dandelions. Fasciations have been described in Pará rubber (*Hevea brasiliensis*) that were propagated by budding (84). Asexual propagation of the Andean oca, *Oxalis crenata* (89), through tubers has already been mentioned. According to DeVries (55), "fasciations occur in so many commercial varieties that they are accessible to everybody; sometimes even the variety owes its name to the frequency of this character, as, for instance, the Swordelder (*Sambucus nigra fasciata*) ; or it may be an almost constant attribute of the cultivated sorts, as in the Japanese spindle-tree (*Euonymus japonica*). When the varieties are either largely or exclusively multiplied in the vegetative way, it is possible that the anomaly, although heritable only to a slight degree, may be manifested frequently, as, for instance, in *Lilium speciosum album corymbiflorum* and in the so-called sword-shaped variety of *Fritillaria imperalis.* Aerial tubers of a fasciated form of *Dioscorea batatas* failed to grow for Bowden and me (19), although those from normal plants grow so readily as to be a weedy pest.

Fasciations induced artificially. Numerous examples of these have been described in a variety of species, including oak (*Quercus rubra*) (70), sunflower (*Helianthus annuus*), Scarlet Runner bean (*Phaseolus multiflorus*) (8, 9, 165, 173), buckwheat (*Fagopyrum esculentum*), snapdragon (*Antirrhinum majus*), the tomato Magnus, *Actinomeris squarrosa, Lythrum virgatum* (89), *Oenothera lamarckiana, Collomia grandiflora,* broad or Windsor bean (*Vicia faba*) (55, 89), cacti (203) and corn cockle (*Agrostemma githago*) (55).

Various methods have been used, one of the most frequent being to amputate the main stem of bean or other seedlings just above the cotyledons. Intentional injury of the growing tip is said to have brought about fasciation in *Ibervillea* (112). Crushing the young stems of the pansy (*Viola tricolor*) (13) and cutting off the root tips of *Vicia faba* (90) have also resulted in fasciation.

At the Missouri Botanical Garden (89), fasciations in *Antirrhinum, Actinomeris, Lycopersicon, Lythrum, Oenothera* and *Collomia* were produced by keeping the plants dry about the time the first flower appeared, only enough water being given to prevent wilting. This resulted in a short flowering period and in an indeterminate inflorescence, the buds near the end of the spike remaining undeveloped. The plants were then abundantly watered, occasionally with manure water. This resulted in numerous fasciations in species of the above-mentioned genera, but was successful only with plants that were in good growth and were well cared for. Apparently no 100% results were obtained by this method, since the experimenter said that, for no apparent reason, "one plant will fasciate, while the next one, of the same species and growing beside it, remains normal". With this treatment Jerusalem cherry (*Solanum pseudocapsicum*), pepper (*Capsicum annuum*), nightshade (*Solanum nigrum*) and a species of *Abutilon* did not fasciate.

Medium X-ray dosage of soaked seed of *Helianthus* (98) induced fasciation in stems and inflorescences. Sunflower, zinnia and tomato seedlings exposed to moderate X-ray doses produced disturbances of the phyllotaxy and fasciation of leaves (95).

Air dry buckwheat seeds (207), soaked for 10 to 60 hours in 0.1% heteroauxin solution, and germinated seeds, immersed in the same solution for 5 to 40 hours, both produced altered phyllotaxy and fasciated branches when grown at a temperature of 25° C.

In greenhouse cultures of sweet peas (*Lathyrus odoratus*) fasciations have been described due to the crown gall organism (23), *Pseudomonas tumefaciens*. Other investigations (146) on such cultures isolated organisms resembling *P. tumefaciens,* but these failed to produce crown gall when inoculated into sugar beets and tomatoes. In these investigations fasciations developed in both the inoculated and non-inoculated controls when the sweet peas were grown from disinfected seed in sterile soil and inoculated with bacteria and fungi obtained from fasciated sweet peas, as well as with *Rhizoctonia solani, Pseudomonas tumefaciens* (raspberry strain) and twelve non-pathogenic bacteria closely resembling *P. tumefaciens.* In this same set of experiments seedlings in sterilized soil remained normal when infested with mealybug larvae and cyclamen mite. Plants grown from disinfected seed in sterilized soil in greenhouse cultures averaged 1.8% fasciations, while similarly treated seed in three-inch pots with crowded roots averaged 18.6% fasciations. No evidence for seed transmission or the crown gall organism as an inducing agent for fasciation was found. A number of well-known sweet pea varieties were used.

Later investigations (114, 115) on sweet peas demonstrated a bacterium isolated from fasciated sweet peas that repeatedly reproduced fasciations on inoculation into sweet pea seedlings. Similar strains of bacteria from a culture standpoint were isolated from leafy galls on chrysanthemums, carnations, and from "cauliflower" strawberry plants, which, on inoculation into sweet pea seedlings, also produced fasciations. At first, seedlings were inoculated by pricking the plumule. This was found unnecessary, since soil inoculation or growing the seedlings in glass bottles in inoculated sand proved effective. The organism is described but could not be identified with any known species. In another study of fasciation in sweet peas the same investigator isolated four new strains of a bacterium, respectively, from *Nicotiana glutinosa, Heuchera sanguinea, Asparagus officinalis* and *Gladiolus,* all of which, inoculated into sweet pea seedlings, produced fasciation. Histological examination and reinoculation of seedlings from previously inoculated plants showed the pathogen to be mainly confined to the exterior of the tissue, although in late stages there was some epidermal cell and intercellular penetration.

It is tempting to interpret the statement made by Hus (89) that "Fasciations may be propagated vegetatively . . . through cuttings, as was done at the Missouri Botanical Garden for fasciations of the tomato, snapdragon", *etc.,* as conveying the idea that his induced types of fasciations in these species were thus multiplied, but I do not think that this is meant. Both of these species have numerous hereditary races, so that it is doubtful that his experimental procedure was the real cause of fasciation, since all investigators agree that certain environmental conditions are more conducive to increasing the degree of expression of this character than are others. I have, however, grown several generations of snapdragon plants from seed of two fasciated individuals which resulted only in normals.

Other than the possibility mentioned above, I have found no record in which artificially induced fasciations have been propagated asexually or otherwise, though it does seem there would be a reasonable probability of it happening, if attempted.

Although DeVries (55, 90) and others believe that all fasciated plants are the result of mutations and that the differences in phenotypic expression are due to latency or unfavorable environments, they emphasize the marked effect that environment plays in overcoming latency. Favorable environmental conditions involve the time of planting, spacing of plants, richness of soil, absence or presence of moisture at certain critical periods, and general weather conditions. However, under the most favorable conditions, so-called poor races never strikingly overlap rich races in percentages of fasciates to normals, and even rich races only approach the 100% fasciation. An example other than those from DeVries is that of flax stem fasciation in Formosa (177). When seed was sown thickly fasciations were infrequent or absent. The greater the spacing, the more frequent the fasciations. When seed is sown monthly in Formosa, plants from the August, September and October sowings have the largest and most frequently fasciated individuals, this being the vigorous growing season. Decline was gradual, tapering off in November and December, to none at all in January to May, the most unfavorable growing period.

Fasciations which have not been investigated experimentally, hence remain unclassified as to transmissibility. Most of the literature records of fasciation belong to this class. Usually a brief description is given, often illustrated. Not infrequently there are surmises as to cause and a statement concerning the number of individuals and the character of the environment.

Some of the most striking examples have occurred in the common dandelion (*Taraxacum officinale*), Australian hoop pine (*Araucaria cunninghamii*), giant Arizona cactus (*Carnegia gigantea*), Viper's bugloss (*Echium vulgare*), *Asparagus officinale,* bull thistle (*Cirsium lanceolata*) (199), sweet potato (*Ipomoea batatas*), *Saxifraga irrigua* (199), Tree of Heaven (*Ailanthus glandulosa*), hog plum (*Spondias mombin*), cultivated petunia, *Pinus resinosa* (105), pumpkin (*Cucurbita pepo*), giant squash (*Cucurbita maxima*) (171), watermelon (*Citrullus vulgaris*), cucumber (*Cucumis sativa*), golden banded lily (*Lilium auratum*), sow thistle (*Sonchus*), cultivated lettuce and other species of *Lactuca,* palms (*Cocos nucifera*), certain orchids, root nodules on legumes and cycads, strawberry and pineapple fruits, and the so-called Wooden Rose or Rose of Hell.

Hard winters, severe pruning, insect mutilation, fungus and bacterial infections (90), unfavorable growth conditions abruptly succeeded by very favorable conditions, nematodes, soil friction, chromosome aberration (99), and highly-manured or very rich soil (55, 90, 107, 186, 197) have been suggested or regarded as causes.

The bulb nematode (*Tylenchus dipsaci*) is believed to be a cause for stem and fruit fasciation in strawberries (182). A warm premature spring followed by a sudden, not-too-cold spell is thought to be productive of dandelion fasciations. Insect mutilation of the young growing tip is a common conjecture involving many species, although adequately proven for very few. In cycads fan-shaped root nodules have been reported as due to *Bacillus radicola.* I have seen similar nodules on the roots of legumes, interspersed with nodules of normal shape. Many observers surmise, without experimental proof, that an hereditary change has taken place. This has been emphasized for the sweet potato, since fasciated vines are found so frequently in widely separated areas. One observer thinks those species with sharp growing points are less liable to fasciation than such plants as asparagus with a round stem tip, since the former are less liable to tip injury from piercing the hard soil surface.

Grafting has also been suggested as a possible cause of fasciation. The cactus *Cereus flagelliformis,* grafted on the stem of another species, *Pereskia aculeata,* grew strongly and in several cases became fasciated. One of these was removed from the *Pereskia* stem and grafted on *Cereus spachianus,* where it "grew into quite a good representation of that mid-Victorian toy—a monkey on a stick" (29). Albino de Souza, a variety of the Old World grape (*Vitis vinifera*), which never becomes fasciated, was grafted on a stock of Goncalo Pires, another variety of this species which is considered to be an hereditary fasciated race, resulting in the former developing fasciation, too (56).

Loss of leaves through spring frosts or June beetle depredations is claimed to have produced fasciation (109), while another investigator (71) says fasciation can be delayed but not prevented by partial defoliation with consequent nutritional disturbances. Fasciations in two species of ash, *Fraxinus excelsior* and *F. ornus,* have had a mite assigned as a cause (90, 134, 197). A species of hawkweed (*Hieracium vulgatum*), DeVries found to be fasciated above a gall produced by *Aulax hieracii,* and normal below it (55). One observer suggested the possibility of an ant's nest as a cause of fasciation in a cucumber vine (86). The same writer mentions examples of fasciation in three widely separated stocks of the chrysanthemum, Thora, and in Winifred, a dahlia variety. Tuber propagation from the dahlia variety produced only normal plants the following season.

Witch's broom is commonly regarded as a form of fasciation. It is especially prevalent in coniferous trees (90, 125, 136) and in the hackberry (*Celtis occidentalis*). In the former case the cause is generally regarded as due to fungi; in the latter case to an insect. Often associated with the witch's broom expression is a typical ribbon-shaped stem fasciation. Such a case occurred in *Pinus resinosa* each year over a five-year period, becoming more severe (105).

CONCLUSIONS

"Fasciation" is a morphological term applied most commonly to an abnormal stem condition in vascular plants, in which the affected regions become flattened or ribbon-shaped (136). All parts of a plant may be altered, many of them in combination with the change in the stem. The two most important characteristics of fasciation are the unregulated increase by weight and volume of tissue over that of the normal type of the same variety or species and the relative loss of control over the growth area, resulting in irregular and unpredictable types of tissue increase. These characteristics are particularly striking when fasciation affects inflorescences or main stem axes. Fasciations are known from over a third of the vascular plant families and probably are potentially a possibility in all of them, with the exception of the wholly aquatic families. Some genera and species are more commonly affected than others. Fasciation has a global distribution but appears to be absent from halophytic and hydrophytic environments.

Anatomical investigations indicate that the various types of fasciation arise from a single growth area and are not the result of a fusion of several such areas. The unbalanced control over such an area results in many irregularities or abnormalities in growth which have much in common with plant galls and cancer. Fasciation may be likened to a generalized gall, resulting from many different causes, and in many cases is indeterminate in growth. As pointed out by Jones, "Plants differ from animals in that there is no migration of abnormal cells to other parts of the organism starting new centers of growth. In plants there is no circulatory system whereby this transfer could be brought about. Fasciations and galls do not show the malignant features characteristic of many tumors in animals". Hence fasciation is not a killing agent.

In some cases fasciation arises as a mutation, the progeny of which all inherit the changed condition in orthodox genetic fashion, and which, generally if not always, express this change under all types of environmental states. Modifying genes apparently greatly affect the expression of the genes for fasciation, as might be expected when such a gene alters so many of the normal character regions.

In other cases fasciated individuals arise through various environmental causes, and they do not transmit this altered state to their progeny, although, once being initiated, apparently the fasciated condition can be transmitted indefinitely through asexual propagation. Phenotypically this transmission expresses itself irregularly, just as it does in gene-based cases, with the result that normal growth shoots appear which superficially, at least in succulent plants, produce a chimeric effect. This effect, though more striking in such plants, is typical of all fasciated individuals and does not necessarily represent a reversion to the normal state. The Mummy pea and the fasciated tobacco races, although arising as mutations, also have normal branches, but no attempt has been made to propagate from them asexually to see whether they would reproduce the fasciated character.

Fasciated succulents, *e.g.*, cristates in cacti and euphorbiads, are always propagated asexually from the fasciated areas, and it is a general belief, though not necessarily satisfactorily proven, that the normal so-called reversions will not reproduce the cristate character. Hence, if this be true, they should be regarded as somatic mutations. Both non-inherited and inherited fasciations phenotypically indistinguishable have been recorded in at least three species. With both the gene-based and the environmentally-caused types of fasciation present in a heterozygous population, with the added complication of modifying gene effects, the difficulty of arriving at the true state of affairs as regards the inheritance of fasciation is easily comprehended.

The basic cause of fasciation is a disturbed metabolism, involving excessive nutriment which mobilizes energy that must be utilized. This energy, once accumulated, must go into growth, and it becomes "wildly" expended in extravagant, abnormal and unpredictable tissue production, generally to the detriment of the plant. Various agencies, innate and external, bring this about.

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ENVIRONMENT AND THE CEREAL SMUTS

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INTRODUCTION

To the late Dr. E. J. Butler is attributed the statement "There are three phases in the history of plant pathology: First the period of de Bary in which the fungus held first place; second, the period in which the host received most attention; and finally, the present period in which disease is considered as an interaction of both under the conditioning influence of environment" (87). The facts presented in this review furnish abundant proof for Butler's observations regarding the present period. Among the cereal smuts it has been well established that their attack may be profoundly affected by various conditions of environment, such as temperature, moisture, type, compactness and reaction of the soil; temperature and moisture of the atmosphere; and length of day. It is known, too, that environmental influences may be operative, not only during the growing season but also during the maturing, cutting, shocking, curing, threshing and storage of the seed grain; in short, during the entire period from one seeding to the next.

Environment ties in also with one of the most active phases of cereal smut investigations, *i.e.,* the determination of physiologic races or parasitic strains of the various species of the causal fungi. At least for the stinking smut of wheat, the reactions of some of the differential host varieties are not the same in all localities. Obviously this could lead to considerable confusion in physiologic race determinations unless the extent of the environmental influences in different localities were appreciated. Also related to physiologic race determinations are the recent findings that the conditions under which a crop matures may be reflected in the succeeding crop in the form of differences in resistance or susceptibility to smut.

For convenience in discussion, each of the 18 cereal smuts occurring in the United States and recognized by Stevenson and Johnson (181) has been placed in one of the three groups noted by Heald (59), *viz.,* the seedling-infecting, the blossom- or floral-infecting, and the shoot-infecting species. Each group is introduced with a brief outline of initial and subsequent infection to facilitate an understanding of how and when the environmental influences operate.

The nomenclature of the cereal smut fungi used herein is that recommended by Stevenson and Johnson (181) based on the International Rules of Botanical Nomenclature.

THE SEEDLING-INFECTING CEREAL SMUTS

In this group spores from smutted heads in the field are blown, washed or otherwise carried to the flowers and developing seeds of healthy heads, as in loose smut (*Ustilago avenae*) of oats, or else the smutted heads are crushed in threshing and the spores are distributed to the mature seed or blown to fields where they mix with the soil and later come in contact with or in close proximity to the host at the time of seeding, as in the stinking smuts (*Tilletia caries* and *T. foetida*) of wheat. After the seed germinates and the seedling grows from the seed to the surface of the soil, the inoculum on the seed or in the soil, or both, develops infection hyphae that enter the seedling and initiate infection (8, 59, 172). The fungus then advances internally through the seedling tissue and, at least in oats, reaches the growing point of susceptible varieties in about 30 days (17, 18, 93). As the plant grows, the infection continues systemically and finally terminates when spores are formed in the young florets of the host, just previous to heading.

Covered Smut of Barley (Ustilago hordei (Pers.) Lagerh.)

The various environmental conditions that have been studied in relation to the incidence of barley-covered smut may be roughly grouped into those exerting their influence before and those after seedling emergence. Relative to pre-emergence factors, Faris (38), in an extensive study of the influence of temperature, moisture and reaction of the soil, dusted seed of Hannchen barley with covered smut chlamydospores, then germinated the inoculated seed in soils at 5, 10, 15, 20, 25 and 30° C. At each temperature the effects of two soil moistures (40% and 50%) and four reactions (pH 5, 6, 7 and 7.8) also were studied. As the seedlings emerged from the controlled soils they were transplanted to a greenhouse bench and then left to grow under usual greenhouse conditions. Regarding temperature effects, percentages of smutted plants in all of the different soils were high at 10°, 15° and 20° C., intermediate at 25° C., and low at 5° and 30° C. Marked differences in smut resulted from seedling emergence in the acid (pH 5) and alkaline (pH 7.8) soils held at 10, 15, 20 and 25° C. and at a 50% moisture-holding capacity. Smut percentages in the acid soil were approximately double those in the alkaline soil. In the neutral soil (pH 7) infection percentages were somewhat higher than those in the alkaline (pH 7.8) soil but far below those in the acid soil. Similar results were obtained at a 40% soil moisture-holding capacity at the 20° and 25° C. temperatures, but at 10° and 15° C. soil reaction produced no marked effects, as it did in the 50% soil.

In another test Faris (38) obtained marked increases in covered smut by varying the soil temperature during seedling emergence. For example, plants that emerged in soil held constantly at 10° C. produced 82.3, 72.6, 50.8 and 42.1% of smutted plants, when the soils during seedling emergence had reactions of pH 5, 6, 7 and 7.8, respectively. The percentages at these reactions, respectively, were 97.9, 97.8, 90.4 and 71.1, however, when the soil temperature was held at 10° C. for six days and then raised to 25° C. over a 36-hour period and finally cooled to 15° C. and held there until the plants were ready for transfer to a greenhouse bench.

In still another study of the effect of soil conditions during seedling emergence on covered smut, Faris (39) germinated spore-dusted seed of Hannchen and Nepal barleys in pure quartz sand and in a rich neutral potting soil, both held at 20° C. The moisture content of the sand ranged from 10 to 90% of the water-holding capacity in steps of 10%, while that of the potting soil ranged from 20 to 70%, also in steps of 10%. After the seedlings emerged both lots were transplanted to a greenhouse bench and grown thereafter under similar conditions. Good to excellent percentages of smut, in most instances, resulted from germination in the potting soil, but low infections generally were obtained in the sand, especially at the higher moisture contents. This is interesting in view of the fact that Reed and Faris (157) found quartz sand quite satisfactory for tests with the smuts of oats and sorghum. Rose (167) reported less smut in barley grown in clay soil than in that grown in sandy, humus or loamy soil. It is not clear, however, whether Rose was dealing with covered or loose smut.

In studies on the influence of date of seeding at Brooklyn, N. Y., Faris (39), in further research on environmental influences, found that the soil conditions became more favorable for infection of winter barleys from early September to late October. Beyond this period the cold soil temperatures became the limiting factor in infection at Brooklyn.

With respect to depth of seeding and related phases, Taylor and Zehner (197) in a four-year test got 2.7 to 115 times as much covered smut in Tennessee Winter barley sown three inches deep as when sown a half inch deep. In Egypt, Jones (83–85) obtained similar results except that high soil moisture during germination constantly reduced covered smut and increasingly so with depth due, apparently, to decreased aeration. In a study of methods of seeding it was found that the Herati method of broadcasting seed on moist ground and plowing it in resulted in six times more barley covered smut than the Afir method of broadcasting on dry land followed by harrowing and irrigation. In studies on prolonging the soil environment through impeding the subterranean growth of the seedling, Tapke (193) obtained distinct increases in barley covered smut by tamping the soil, by increasing the depth of the soil layer above the seed and by using heavy soil. Woodward and Tingey (220), however, reported that in Utah depth of seeding and date of seeding produced variable results but had little consistent influence on the percentage of smutted heads.

Until recent years barley covered smut had been an unusually difficult smut to understand. Supposedly the spores remained enclosed in the smutted heads until threshing when they were distributed to the exterior surfaces of the seed. This idea was difficult to accept, however, because seed surface disinfectants, like copper carbonate dust, usually failed to effect control in naturally inoculated seed from smutted fields. Moreover, the artificial inoculation of clean seed by blackening the surface with millions of spores usually failed to produce high percentages of smutted heads, even in susceptible barleys. It is known now that the past difficulties with this smut were due chiefly to a lack of understanding of the influences of environment on infection. An important lead was the outstanding contribution by Zade (224) in 1922. He found that, at least under the humid conditions in his area in Germany, spores of the oats loose smut (*Ustilago avenae*), which come in contact with the exposed oat flowers at pollination time, normally do not remain dormant, as long believed, but germinate by producing sporidia and mycelia that result in an effective load of inoculum inside the hulls near the vital point of attack. This discovery opened the way to the solution of barley covered smut problems following artificial seed inoculation or seed treatment with copper carbonate dust. Mackie (109) stated that under California conditions thorough and repeated dusting of the seed with copper carbonate each year appeared to control covered smut satisfactorily. In more humid areas of the country, however, copper carbonate repeatedly has proved too ineffective to warrant its recommendation (78, 101). Climatic conditions are largely accountable for this situation, as shown by Tapke (194) in experiments in humid Virginia. He found that spores of *U. hordei* may be actively disseminated from heading to threshing, and that, like those of *U. avenae,* they do not remain dormant after reaching the seed but frequently germinate during the maturing, cutting, shocking and storage of the grain. This results in the production of a subhull mycelium which is commonly found in the naturally inoculated seed of humid areas, and which is protected by the hulls against the fungicidal action of copper carbonate dust. In fact, in one experiment spores dusted on the surface of barley seed appeared not only to germinate but to send mycelium beneath the hulls under humid conditions of storage. At least the copper carbonate seed treatment was 100% effective in control when applied to the inoculated seed after arid conditions of storage but relatively ineffective after humid storage. This understanding of the role of environment under natural conditions led to the development of an effective artificial method of seed inoculation (196), but investigators still were at a loss to understand such contrasting results as those of Briggs (20) and of Faris (38, 39), for example, both of whom used seed superficially dusted with chlamydospores. Briggs got practically no covered smut in seedings at two places in California from seed of 570 varieties and strains of barley in 1919–20 and obtained similar results with 100 varieties the following year. On the other hand, Faris, as noted above, got excellent results with superficially inoculated seed over wide ranges of soil moisture, temperature and acidity. It now appears that these differences likely were due not only to different preemergence conditions affecting the initial infection but also to different post emergence conditions affecting the subsequent infection. Briggs' tests were conducted in the field, while those of Faris were conducted in the greenhouse. Recent studies have shown that greenhouse conditions during the post emergence growth of barley plants are especially favorable for infection and subsequent development of a heavy outbreak of covered smut. In studies reported by Tapke (191) seedlings of two winter barleys were transplanted from small pots to an outdoor bed and to a greenhouse bench at Arlington, Va., in October, after emergence indoors under controlled soil conditions favorable to infection. When the seed was inoculated by the spore-suspension method, whereby chlamydospores are deposited on the caryopsis underneath the hulls and the fungus has immediate access to the seedling tissue as soon as sprouting begins, high and practically equal amounts of covered smut developed in the plants that went through the winter outdoors and those that were protected against cold and other climatic vicissitudes in the greenhouse. However, when the spores were placed outside the hulls by dusting the exterior surface of the seed, as in the tests of Briggs (20) and Faris (38, 39), covered smut was (*a*) relatively low (27.8%) when the plants were placed outdoors immediately after emergence, (*b*) appreciably increased (55.1%) when the seedlings were kept in the greenhouse two weeks after emergence before transplanting outdoors, and (*c*) further increased when the seedlings were held four weeks in the greenhouse before transplanting outdoors, (65.3%) or grown continuously indoors (63.9%). It appears, therefore, that the method of inoculating seed by superficially dusting the hulls with smut does not approximate the natural method of inoculation in humid areas and that it is not intrinsically an effective method. Under greenhouse conditions, however, it appears to be effective, due to an environment unusually favorable to infection in the post-emergence period. It is a common observation that the incidence and severity of smut attacks in the greenhouse usually exceed those in the field.

An example of how subtle the effects of soil environments can be is well shown by the inconsistent data that have been reported in studies on the effects of dehulling naturally inoculated seed. Following Zade's (224) discovery noted above, that the effective inoculum of the oats loose smut fungus (*Ustilago avenae*) consists of mycelium enclosed by the hulls, and the later discovery of a similar situation with the covered smut of barley (*U. hordei*) (194), a question arose as to whether this mycelium was located chiefly in the inner parenchymatous lining of the hulls or in the outer skin or pericarp of the caryopsis. Since these layers are adjacent or contiguous, the location of inoculum in one or the other would seem to make little difference. However, with oats, as noted in detail later, several investigators sought to answer the question by sowing seed with the hulls intact or removed from the caryopsis by hand. Presumably the reduction in smut in plants from the dehulled seed would be a measure of the proportion of inoculum removed with the hulls. In a study of the effect of dehulling in naturally inoculated barley, Tapke (194) sowed six lots under field and greenhouse conditions in Virginia and under field conditions in New York. The results were erratic. Three of the lots from which the hulls were removed, for example, produced a decrease of smut under some conditions and an increase of smut under others. Similar data have been reported in tests of oats (190). It is, therefore, evident that although the removal of hulls obviously does not change the soil environment, it nevertheless changes its effect through exposing the naked caryopsis and the inoculum that it bears to direct contact with the soil conditions and the soil organisms. Due to the idiosyncrasies of different soil environments and their various effects on the portion of inoculum that resides in the pericarp of the caryopsis, removal of the hulls seems hardly a reliable method for determining whether inoculum in the hulls or pericarp is chiefly responsible for the initial infection of barley and oats by *U. hordei* and *U. avenae,* respectively.

Black or False Loose Smut of Barley (Ustilago nigra Tapke)

This second loose smut of barley was discovered in the United States only 15 years ago (188), but recent studies have shown that it is widespread and important in this country (195), Canada (6, 30, 31) and Palestine (123).

Observations by Oort (141) indicate *Ustilago nigra* also may occur in Holland.

It is commonly called the "false" loose smut of barley in Canada. In the United States it has been variously referred to as the "black", "nigra", "intermediate", "false" or "shallow-borne" barley loose smut. *U. nigra* is a floral-infesting, seedling-infecting loose smut, practically identical with the long-known oats loose smut (*U. avenae*), except that it attacks barley.

In studies by Leukel (100, 102) soil temperatures during seedling emergence of 5° and 30° C. were found generally unfavorable, 10° and 25° C. fairly favorable, and 15° and 20° C. optimum for smut development. Varying soil temperatures also have been found effective in causing pronounced differences in smut attack. Leukel (102) reported that plants grown to emergence in soil at 30° which was then permanently changed to 13° C. showed less smut than the plants kept continuously in 30° soil. Similar results were obtained with changes of 13° to 30° C. and 13° to 5° C., but significant increases in smut were brought about by changes of 5° to 13° and 5° to 30° C. In studies along similar lines Josephson (86) found that changes from the low (5° C.) to the optimum (15–20° C.) or high (30° C.) soil temperature and from the high to the optimum, increased smut, while changes from high to low and from optimum to either high or low, decreased smut.

Soil moisture in the pre-emergence period did not give consistent results as a factor influencing smut development in one study (86), but in another study (102) soil with a high percentage of saturation (85–90%) was generally inimical to good infection, especially at 5° and 30° C., while relatively dry soil favored infection at 5° C. At other temperatures soil moistures from 30 to 55% saturation had little influence on the incidence of the *"nigra"* smut. Likewise soil reaction (102) over a range from pH 3.8 to 8.1 failed to show any consistent effects on the development of this smut in barley plants.

In line with results with barley covered smut (*Ustilago hordei*) and oats loose smut (*U. avenae*), Tapke (191–193) found that cold conditions after the seedlings have emerged may result in marked smut reduction. Temperate greenhouse conditions continuously after emergence, or for 10 to 30 days, followed by exposure to outdoor conditions, resulted in decided increases in smut in comparison with that developing in plants exposed to outdoor cold beginning immediately after emergence.

In view of the similarity of *Ustilago nigra* and *U. avenae* it might be expected that much of the fuller knowledge of the latter relative to environmental influences may later be found to apply also to *U. nigra.*

Loose and Covered Smuts of Oats (Ustilago avenae (Pers.) Rostr. and U. kolleri Wille)

Much of our information on the environmental influences in the pre-emergence phase of oats inoculated with covered smut has come from the extensive investigations of Reed and Faris (157). Seeds of *Avena nuda* var. *inermis* and *A. sativa* var. *Victor* were inoculated with covered smut (*U. kolleri*) and grown to seedling emergence in sand at eight different moistures (15, 20, 25, 30, 35, 40, 50, and 60% of the water-holding capacity) at each of six temperatures (5°, 10°, 15°, 20°, 25° and 30° C.). The influence of seven different soil reactions ranging from pH 4.6 to 8.6 in sand at 19°–22° C. and 20% moisture also was studied. Including all moistures at each temperature, the results at 5°, 10°, 15°, 20°, 25° and 30° C. were 32.1, 49.1, 71.2, 70.0, 83.2 and 18.2% of smutted plants, respectively, for *A. nuda,* and 3.3, 25.9, 37.4, 57.3, 63.8 and 20.5% for *A. sativa.* In both varieties, therefore, best results were obtained at 25° C. In *A. nuda* infections occurred over a range of soil moistures from 15 to 80%, while in *A. sativa* the range was 15 to 60%. In both varieties the lower moistures proved most favorable for smut. Relative to soil reaction, that near the neutral point or slightly acid during seedling emergence promoted the highest percentages of covered smut in these oats.

A point of special interest brought out by Reed and Faris (157) is the interdependence of soil moisture and temperature effects on smut. For example, 15° C. was optimum for infection of *A. sativa* at 15% soil moisture, while at 20 and 25% moisture, 20° C. was optimum, and 25° C. was optimum at still higher soil moistures. It was emphasized, therefore, that the term "optimum temperature for infection" is without significance unless due regard is paid to other possible influencing factors. This may explain Traen's (206) finding that 15° C. rather than 25° C. was most favorable for covered smut in oats. He likewise notes that during seedling emergence, soils of low moisture content are far more favorable to infection than soils high in moisture.

In a study of oats loose smut (*Ustilago avenae*), similar to that of Reed and Faris (157) with covered smut, Bartholomew and Jones (14) also used the highly susceptible *Avena nuda* var. *inermis* but germinated the seed in soil instead of sand. In soil of 36% saturation at temperatures of 8°–29° C. they obtained a minimum of 50% smutted plants, with 100% at 18°, 21° and 23° C. At similar temperatures with 67% saturation much less smut occurred, and there was still less with 80% saturation. At all moistures a temperature of 18°–22° C. was most favorable for infection. As frequently noted in studies of this kind, the high moisture–high temperature combinations were more inimical to smuttedness than the low moisture–low temperature combinations. With 60 and

80% soil moistures at 31°–32° C., for example, no smut occurred, but at 8° C. with 36% soil moisture, 67% of the plants were smutted, and at 10°–14° C. with 30–35% soil moisture, 96.5% became smutted by *U. avenae.*

In 1908 Falck (37) reported what now appears to have been a most important discovery, but its significance was not appreciated at that time. Falck examined oat flowers after blossom inoculation and found that some of the spores on the stigmas had germinated. He also found mycelium penetrating the outer layers of the caryopses of seeds from inoculated flowers. Despite this observation it was believed up to 1922, on the basis of the pioneering studies of Brefeld (19), Jensen (77) and Kellerman and Swingle (88), that spores on oat seed remained dormant from the time of contact until the seed was sown the following season and began to germinate. In 1922, however, Zade (224) reported the rediscovery of spore germination in the oat flowers and confirmed it in subsequent studies (225, 226). When he applied spores of *Ustilago avenae* to the flowers of oats at pollination time, almost all of the spores that fell on the stigma began to germinate in 15–20 hours under the mild damp weather conditions at the time of his inoculations. The promycelia from the spores gave rise to sporidia that budded and produced mycelium in the parenchyma and epidermis that lined the inner surface of the glumes. The load of inoculum was thus substantially increased at or close to the vital point of attack. From the economic point of view especially, this is a factor of prime importance, and in Zade's opinion explained the frequent epiphytotic attacks of the disease and the relative ineffectiveness in smut production from spores on the exterior of the hulls. In further studies by Arland (10), Diehl (34) and Roesch (166), three of Zade's co-workers in Germany, and by Sampson (170) in Wales, and Gage (49) in the United States, it was found that weather conditions following pollination may appreciably affect the germination of spores enclosed within the glumes. In general, in cool wet weather the spores form tubes and sporidia, but if it is warm and dry they form mycelium directly, while in very dry years spores on the ovary remain ungerminated. Gage (49) calls attention to the fact also that in damp cool weather oat blossoms often fail to open at pollination, thus excluding the entrance of spores. Through its influence on the positional relation of inoculum to infection, this factor might well be important in the seasonal differences in smut prevalence and damage to oats. Gage (49) found also that spores on the surface of seed in petri dishes may germinate under conditions of moisture and temperature too low to support germination of the oat seed. Taking advantage of the low moisture differential in favor of the pathogen, Tamme (185) injected several drops of spore-charged nutrient solution between the glumes and caryopses of oats. The inoculated seed then was kept in petri dishes with moisture sufficient to germinate the spores but not the oats. Plants from this seel were 60% smutted. In contrast with the above investigations, however, McKay (116) and Kitunen (91) were unable to attach much importance to subhull mycelium and concluded that spores enclosed by the hulls or on the exterior surface of the hulls are the important agents in the initiation of infection during the subterranean emergence of the seedling following seeding. Despite these differences of opinion, the investigations of Zade (224–226) and his followers threw a new light on the activities of the oat smut fungi during their incubation period between inoculation and seeding and on the importance of environmental influences during this period. Moreover, as noted previously, these findings on oats opened the way to similar discoveries on the prime importance of environmental factors in an understanding of the life-cycle and control of the barley smuts (194).

Out of the work on infestation of oat flowers and seeds with spores and mycelium of *Ustilago avenae* and *U. kolleri*, differences of opinion have arisen as to whether spores that lodge within the glumes of oats send their mycelium mostly in the tissue that lines the inner surface of the glumes or in the adjacent epidermis of the caryopsis or seed proper. The different results may be largely a reflection of the environmental conditions under which the experimenter worked. For example, Gage (49) grew oats in a greenhouse environment very favorable for infection and found that removal of the hulls of seed from hand-inoculated flowers effected only a small reduction in the percentage of loose smut. He concluded, therefore, that the effective inoculum resides chiefly in the pericarp of the caryopsis. However, under field conditions relatively unfavorable for loose and covered smut, Johnson (80) obtained high reductions of smut in five naturally inoculated lots as a result of seed hulling. He concluded, therefore, that most of the inoculum was carried on or in the hulls and that it was removed with them in the process of hulling. In a further study by Tapke (190) seven to ten lots of oats naturally inoculated with loose smut spores were grown from seed with the hulls on and with the hulls removed, under field and greenhouse conditions in Virginia and under field conditions in Idaho. The results showed that hulling is not a reliable basis on which to determine whether inoculum in the hulls or pericarp is accountable for most of the loose smut in oats. The different environmental conditions in the three locations of the experiment so markedly influenced the range of reduction in smut due to hulling that inoculum either in the hulls or pericarp could have been held accountable for most of the infection. Within individual lots the reduction in loose smut from dehulled seed varied as widely as from 2.1% to 90.2% in the three locations of the experiment.

In recent studies on loose and covered smuts of oats, Tervet (199–201) has compiled interesting data showing the remote effects of environmental influences. The lots of different varieties of oats grown in one locality in different years or in different localities during a single year showed great variation in amounts of smut from uniformly inoculated seed. Variations within a variety were as great as those found between certain varieties. These variations were not genetic but apparently due to modifications in the seed resulting from the different environments under which the various lots were produced. The results indicate the importance of a uniform environmental background for varieties used as differential hosts in studies of physiologic races of the oat smut fungi.

In the score of years from 1890–1910 a number of pioneering studies appeared that dealt with the influence of temperature on the germination of oats, oat smut spores and infection, and on the influence of date of seeding on smut in oats. *Ustilago avenae* usually was the smut under observation. Hecke (61) recorded the cardinal temperatures for the germination of oats as minimum 4°–5°, optimum 25° and maximum 30° C., and for germination of the smut spores as 5°–11°, 22°–30° and 30°–35° C. Similar results for spore germination also were recorded by Herzberg (62). Apparently the first to study the temperature-infection relation, Brefeld (19) inoculated oats with conidia and obtained infections of 40–60% and 27–30% at germination temperatures of 7° and 15° C., respectively. In a series of pot experiments, von Tubeuf's (208) results were 1% infection from inoculated seed germinated at 1°–9°, 12–20% at 10°–20° and 22–24% at 20°–21° C.

Relative to dates of seeding, Kellerman and Swingle (90) found no marked differences in the amount of smut in a number of seedings between March 22 and April 12. In a group of oat varieties sown on May 4, Jones (82) found a moderate amount of smut, but none appeared in plants from the seed sown on July 13. Hiltner (64) recorded a progressive increase in the amount of smut in seven seedlings from March 17 to May 21, while Clinton (28), Ravn (152), von Tubuef (209) and Heald (58) are in accord in finding the most smut from midseason seedings and lesser amounts from early and late seedings. Clinton (28) also reported that deep seeding favors smut. Plants from seed sown at depths of four inches, two inches and one inch developed 10, 6, and 2% smut, respectively.

In a study of the influence of growth of the host on smut development in oats, Reed (154, 155) used four varieties, each inoculated with three physiologic races of the covered smut and three of the loose smut. The plants were subjected to four treatments, receiving both sodium nitrate fertilizer and evening illumination, or one or the other alone or neither of the two. Although the treatments resulted in wide differences in growth of the host plants, the conclusion was reached that no significant effect on the development of the oat smut was brought about.

The Loose and Covered Kernel Smuts of Sorghum (Sphacelotheca cruenta (Kuehn) A. A. Potter and S. sorghi (Lk.) Clint.)

Much of our information on these two smuts relative to environmental influences during the pre-emergence period is attributable to the investigations of Reed and Faris (156, 157). They sowed inoculated seed of a number of varieties of sorghum in soil at 12° to 37.5° C. and 10 to 80% moisture. Attention was given also to the influence of soil reaction. The conditions desired during seedling emergence were accurately controlled and maintained through automatic instruments. Following emergence, seedlings were transplanted to the field and left to grow there under the conditions that prevailed. The results with *Sphacelotheca sorghi* and *S. cruenta* were similar. Relative to temperature, the range for infection was wide, some occurring at 15° C. in all tests and at 35° C. in nearly all. The range for maximum infection also was wide. In one experiment, for example, the pre-emergence temperature which resulted in the most smut was 15° C. with Blackhull kafir, 20° C. with Valley Kaoliang and 25° C. with Red Amber sorgo. High percentages of infection also were secured over wide ranges of soil moisture and reaction, but the lower soil moistures and the slightly acid soils were most favorable. Reed and Faris (156, 157) emphasized the point that "soil moisture, soil temperature and soil reaction are interdependent factors. Their interaction determines whether infection will take place and also the severity of the attack. Any one of these factors may be a limiting one in the prevention of infection. The term 'optimum temperature for infection', for example, is without significance unless due regard is paid to other possible influencing factors".

Reed and Faris (156, 157) also called attention to the common notion that there is a definite relation between the occurrence of smut in cereals and the rate of subterranean growth from seeding to seedling emergence. They studied, therefore, the influence of different soil conditions on the rate of seedling emergence and this in turn on the incidence of loose and covered kernel smuts in sorghum and covered smut in oats. The conclusion was reached that although different soil conditions may greatly influence the amount of smut, the relative degree of susceptibility of each variety is inherited and not dependent upon difference in the rate of seed germination.

In their study of sorghum smuts and varietal resistance in sorghums, Reed and Melchers (158) note that infections with covered kernel smut at Columbia, Mo., in 1918 were markedly lower than those of previous years. This was due, likely, to the high temperature and low precipitation before and after seeding in that year.

In a comprehensive field study of environmental conditions at planting time on the incidence of the covered kernel smut (*Sphacelotheca sorghi*) at Manhattan, Kansas, Melchers and Hansing (117) sowed inoculated kafir seed at weekly intervals from shortly after the middle of March until the latter part of July. The test covered the five-year period 1932 through 1936. Full records were made of soil moisture, temperature and acidity, air temperature and rainfall.

The conclusions from this field study corroborated those of Reed and Faris (156, 157) under controlled conditions in the greenhouse. Melchers and Hansing (117) found that heavy infection occurs at any temperature below 75° F. (23.89° C.) that allows the seed to germinate, that an absolute minimum temperature of 28° F. (minus 2.22° C.) during the emergence period is no hindrance to infection, that mean maximum soil temperatures of 75° F. (23.89° C.) or above during the emergence-infection period result in smut reductions and that soil moistures of 28% or more, on the dry basis, reduce infection considerably, even though favorable soil temperatures prevail. In general, medium to low soil moistures and temperatures were most conducive to maximum infection, high infections were consistently associated with low temperatures and fair infections occurred over a very wide range of soil moistures and temperatures.

Using the technique of seed germination of Reed and Faris (156, 157), Marcy (112, 113) germinated sorghum seed, inoculated with *Sphacelotheca sorghi,* in approximately neutral sand at 18–23° C. and at moisture contents of 30, 20 and 10% and also at 10% supplied as a 2% sucrose solution. The latter was the most favorable and caused Feterita to exhibit pathological effects of infection by *S. sorghi* not noted under the other conditions. The infection was characterized by a complete or partial blasting of the heads, with the production of occasional smut balls, and by excessive tillering and branching.

In India, where sorghum is one of the leading crops and the damage from smut, especially covered smut, is often very severe, Kulkarni (94) found that 20°–23° C. is the optimum range for germination of the spores of *Sphacelotheca sorghi* and *S. cruenta,* that for the seed is 36°–40° C., and infection is most likely to occur at temperatures between 16° and 30° C. In a further study (95) he notes that loss from *S. sorghi* is especially severe in Bombay, Madras, Central Provinces and Burma where the temperature at sowing time is between 21° and 30° C., a range favorable to good spore germination. In the Indo-Gangetic plains, however, where the temperature at sowing time is between 30° and 40° C., a range too high for good spore germination, *S. sorghi* is relatively rare. In order to test the assumption that the distribution of the smut might be related to soil temperatures at sowing time, Kulkarni germinated inoculated seed in two pots, one held at 25° and the other at 40° C. for three days after sowing. On the fourth day the seedlings were transplanted to large outdoor tubs and left there to mature. At 25° C. 35 of 64 plants were smutted, but at 40° C. all of the 69 plants were smut-free. Similar results were obtained in field experiments in different localities with different temperatures at sowing time. Kulkarni (95) concluded "that temperature is the controlling factor in the distribution of the grain smut in India". He notes also, relative to soil moisture, that the seed bed may be just damp enough to allow the spores to germinate while insufficient for the sorghum. In this case the germ tubes perish before infection can take place. When the moisture is more than sufficient for both the fungus and the host, the germination of seed is so rapid that the young seedling may outstrip the germinating spores.

In a recent study on covered smut, Leukel (104) sowed inoculated seed of Leoti sorgo in three separate lots of Keyport silt loam adjusted to 30, 50 and 70% saturation. Infection percentages of 26.8, 81.6 and 5.6, respectively, were obtained. These results, as noted by Leukel, "show a very pronounced relation between relative soil moisture and infection by smut in the plants from untreated seed".

Vaheeduddin (212) sowed inoculated seed from early May to early June during two years at St. Paul, Minn., and found that early seedings under cool conditions were less favorable to *Sphacelotheca sorghi* than were soil temperatures between 20° and 25° C. during seedling emergence. Isenbeck (75) obtained similar results. According to Takasugi and Akaishi (184), the incidence of loose kernel smut was found to be promoted by piling up soil over the seed and by a deficiency of potassium and phosphoric acid in the fertilizer.

Bunt or Stinking Smut of Wheat (Tilletia caries (DC.) Tul. and T. foetida (Wallr.) Liro)[3]

Numerous references are available that deal with environmental influences on the incidence of stinking smut or bunt in wheat. The previous reviews of much of the literature by Woolman and Humphrey (221) and Holton and Heald (67) have facilitated preparation of the present work.

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[3] The International Rules of Botanical Nomenclature require that the names *Tilletia caries* (DC.) Tul. and *T. foetida* (Wallr.) Liro be used in preference to *T. tritici* (Bjerk.) Wint. and *T. foetens* (Berk.) Trel. or *T. levis* Kuehn, respectively. Accordingly, this usage is followed.

Temperature. Prevost (149) in 1807 first noted the influence of this factor on spore germination in *Tilletia.* Heuser (63) reported the cardinal temperatures for the germination of wheat as 3°–4.5°, 25° and 30° C., those for the bunt spores as 5°, 16°–18° and 25° C., while 6°–10° C. favored high infections. Along similar lines Mouravieff (126) found the best temperatures were 20°–25° C. for germination of the wheat, 10°–15° C. for germination of the spores, and 5°–10° C. for infection.

The results of a comprehensive study of the influence of soil temperatures and other factors on the incidence of bunt were reported in 1924 by Faris (40). In one test, seed of two winter wheats was inoculated with a collection of *Tilletia caries* and one of *T. foetida* and sown in soil accurately maintained at 5°, 10°, 15°, 20°, 25° and 30° C. in constant temperature tanks. When the seedlings reached the second leaf stage they were transplanted to a field in autumn and left to grow and go through the winter under natural conditions. A similar test was conducted the following spring with two spring varieties and another selection of one of the winter varieties (Dawson) used in the autumn test. In the spring test each variety was inoculated with two collections of each of the two bunt fungi. With *T. caries* only low infections were obtained in the winter wheats sown in the autumn, but with *T. foetida* high percentages of bunt resulted when the preemergence temperatures were 15° C. and below, the highest occurring at 5° followed closely by those at 10° and 15° C. A rapid decline set in at 20°, and only negligible amounts of smut occurred at the 25° and 30° C. temperatures. The percentages of bunt in the winter wheat that was sown in the spring were, as stated by Faris (40), "remarkably close to those obtained in the fall planting with another selection of this variety". The spring varieties in the spring test reacted somewhat differently. High percentages of infection again were obtained at 5° and 10° C., but the first rapid decline set in at 15° rather than at 20° C., as in the winter wheats. This decline continued at 20° and no smut occurred at 25° and 30° C. Another feature of the spring test was that one of the varieties, Marquis, consistently produced higher infections at 10° than at 5° C. with all four smut collections, whereas the other spring variety, Red Fife, reacted in general like the winter varieties in producing the highest infections at 5° C. These results indicate that the variety of the host and the species of the parasite may be factors in determining the optimum temperature for the infection of wheat by *T. caries* and *T. foetida*.

In experiments with *Tilletia tritici* by Rabien (150), seed was inoculated, then incubated at 10° C. for different periods. Lastly the material was transferred to a greenhouse held at 20°–22° C. Plants from seed incubated three, four, five and eight days developed 0.7, 4.1, 6.3 and 40% bunt, respectively. Incubations at 5° C. for less than nine days, followed by transfer to the greenhouse, resulted in only a trace of bunt, but up to 49.1% was obtained from longer incubations.

In studies conducted by Rapin (151), tests were made in the field at a soil temperature range of 2.4° to 8° C., also in steam-heated beds around 15° C. and in a greenhouse around 27° C. The percentages of bunt were 33 in the field, 50 in the beds and 1.3 in the greenhouse. The low amount in the greenhouse was explained as due to the retarding effect of high temperature on the infection of wheat by *Tilletia caries*.

In four varieties of wheat germinated at 7°–8° C., Munerati (131) obtained 92–100% infection but only 1.4–12% when the varieties were germinated at 18°–20° C. In a later report dealing with the influences of both constant and variable soil temperatures, Munerati (132) got only smut-free plants when grown for five weeks in soil at 2°–4° C. or at 20° to 25° C., and then left to mature in the field. Plants grown at the low range for 20 days, then at the high range for the remainder of the five weeks, also were smut-free. Plants held at 10°–12° C. had 15.2% bunt, while those held at 2°–4° C. for 20 days, then at 10°–12° C. for seven days and finally at 22°–25° C. for the remainder of the five-weeks period, had 24.7% bunt. The two varieties used in the test reacted similarly.

According to Crepin *et al.* (32), a soil temperature between 5° and 12° C. at sowing time and during the three or four following weeks is optimum for bunt infection. Average humidity and well-aerated soil also favor infection.

In another phase of temperature relations, Defago (33) reported that in culture *Tilletia caries* grows best at 21°, while 18° C. is best for *T. foetida*. This fact, according to Defago, may explain why *T. caries* is prevalent in warm countries and in continental countries where spring wheat is grown almost exclusively.

The effect of high temperatures at heading time on the occurrence of bunt was reported in 1939 by Sempio (176). The conclusion was reached that infected plants must remain exposed to a temperature of 32° C. for at least five consecutive days before *Tilletia caries* is definitely arrested.

Ciferri (27) attributes the absence of *Tilletia caries* and *T. foetida* in southern and insular Italy to the relatively high spring and autumn temperatures and to the high resistance of *Triticum durum* and *T. turgidum* wheats grown there.

Infection Temperatures and Bunt Epidemics. The association of epidemics of bunt and temperatures favorable to infection during seeding and seedling emergence has been noted by Reichert (160) and Mouravieff (126). According to the latter, severe outbreaks of bunt occurred in the Ukraine in 1922 and 1927. In these two years the temperature curves during the sowing period were identical and lower than the average for the six-year period, 1922–1927. Only light attacks occurred in the years between the epidemics. Similarly Reichert (160) reported high percentages of bunt, ranging from 24.8 to 66.6% in his 1924 plantings coincident with a prevailing low temperature of 10.6° C. during seed germination. This temperature is favorable to infection. In 1925, however, a less favorable temperature, 15.2° C., prevailed during germination, and the percentages of smut ranged only from 7.1 to 22.

Varietal Differences in Response to Temperature. Papers in 1932 by Feucht (42) in Germany, and Smith (179) in the United States, confirmed previous indications by Hungerford (72) and Faris (40) that wheat varieties may differ in their response to temperatures for optimum infection. In Feucht's (42) studies, the optimum (5°–6° C.) for Strube's Dickkopf wheat, for example, was not the same as that of other varieties. Furthermore, Hope wheat, as shown by Smith (179), is completely susceptible to one race of *Tilletia caries* when grown continuously at 9° C. but resistant to this race when grown at 9° C. to emergence and then transferred to a 20° C. temperature. The Jenkin variety, however, is susceptible under both conditions. Feucht (42) noted also that low soil and air temperatures at seeding time promoted bunt with both species but that very low or very high temperatures during this period reduced its incidence.

Soil Moisture. In studies dealing chiefly with soil moisture in relation to bunt, the experiments of Jethro Tull in 1733 (210) are doubtless the earliest. He transferred wheat seedlings from a field to the window of his chamber where they finished growth in very wet soil, a part of the roots being in water continuously. These plants were entirely bunted, while plants which remained in the field were rarely bunted. Tull, therefore, concluded that excessive soil moisture promotes bunt. In other early studies, von Tubeuf (208, 209), Ivanoff (76), Volkart (215, 216), Malkoff (111) and Appl (9) cited observations or experiments that indicated an importance of soil moisture in the incidence of bunt.

In the later experimentation Hungerford and Wade (73) reported that little bunt developed when the soil moisture content was as low as 7%, but a gradual increase in infection occurred as the moisture content increased up to 38%. Hungerford (72) later confirmed these results and concluded that the infection of wheat by the rough-spored bunt (*Tilletia caries*) is favored by soil temperatures ranging from 9° to 12° C. and a soil containing 22% moisture with a moisture equivalent of 20.7.

Rabien (150) sowed inoculated seed in soils of 20, 40 and 80% of moisture capacity and obtained bunt percentages of 22.3, 55.3 and 10.7, respectively. He concluded that the low infection at the high moisture was due to rapid germination of the wheat under conditions that retard spore germination due to a reduction in oxygen supply.

According to Finnell (43), a definite relation exists between soil moisture and the incidence of bunt. The percentage of bunt was highest in his tests when considerable moisture was present in the soil at germination. In Argentina Nieves (137) found that conditions most favorable for infection of wheat by *Tilletia foetida* occur in the autumn. The highest infection was obtained when the seed germinated in soil that became progressively drier in the absence of rain. A regular though slight rainfall during the seed germination period proved detrimental to high infection. The least smut was obtained at soil temperatures of 6° to 7° C. Field observations by Djelaloff (35) indicated there was less bunt in irrigated than non-irrigated wheat and that the severity of attack also was reduced by good tillage.

In studies involving artificially inoculated soil, Martin (114) found that percentages of bunt were appreciably higher when wheat was sown a week or ten days after the soil was inoculated and watered than when it was sown on the day of inoculation and watering. This was due, presumably, to the inhibiting effect of the saturated soil on spore germination in the early seeding.

Moisture and Temperature. Relative to investigations dealing with the influences of both soil moisture and temperature on bunt, Woolman and Humphrey (222) reported in 1924 that the optimum soil moisture content for infection by *Tilletia caries* in the basaltic soil of the Palouse country lies between 16 and 30%. The optimum temperature range for spore germination was 18° to 20° C. Wheat sown in soil above 20° C. was practically immune from infection by seed-borne spores, but this was not necessarily true of soil-borne spores.

Gibs (53), in tests with both of the bunt fungi, obtained the highest infections in soil at 9°, less at 4° and the least at 17.7° C. He assumed that the relative speed of germination of smut spores and wheat kernels was such that the host seedlings were able to escape infection at 17.7° C. despite the fact this temperature was optimum for spore germination. It was further assumed that at 9° C. germination of the spores was rapid relative to that of the seed, thus accounting for the high infections at this temperature, while at 4° C. both spores and seeds germinated so slowly that ample time for good infection was provided. Also noted by Gibs (53) was the fact that a low soil moisture content promoted greatly increased percentages of bunt in all varieties at each temperature tested.

In studies recorded by Leukel (103), soil temperatures of 6° to 15° C. from sowing to seedling emergence were optimum for infection by *Tilletia caries* and *T. foetida*. The highest average infection by both species occurred at 10° C. Temperatures below 5° and above 20° C. were highly unfavorable. Relative to soil moisture, the percentage of infection in plants from inoculated seed was reduced from 31.4 to 6.5 by saturating clean soil and from 54 to 28 by saturating spore-infested soil. These reductions are attributed by Leukel to the fact that in wet soils the oxygen supply is insufficient for abundant spore germination. Olgyay (140), also reporting on soil moisture and temperature influences, notes that infection was high in seedings at 10° to 18° C. and low at 4° to 10° C. and at 18° to 22° C. Below 4° and above 22° C. no infection occurred. The incidence of bunt also was reduced in very dry soil and when the soil moisture exceeded 40 per cent.

Post-emergence Environment. A few investigators have studied the outbreak of bunt as influenced by various environmental factors after the plants appear above ground, *i.e.,* after the period of initial subterranean infection during seedling emergence. Faris (40) germinated inoculated seed of two winter wheats under controlled conditions favorable for infection, then divided the seedlings at random into two lots. One was transplanted to a greenhouse, the other to a field in autumn. The latter passed through the severe cold of winter, while the greenhouse plants made uninterrupted growth from germination to maturity. Despite these wide differences in post-emergence environment, the field and greenhouse plants reacted similarly to bunt, both contracting high percentages of infection. Caspar (24) found that *Tilletia caries* and *T. foetida* were more severe on moderately damp soil than on very dry or very wet soils, that shading increased infection by prolonging the period of susceptibility and that late sowing of spring wheat and warm weather following the sowing of winter wheat enabled the plants to outgrow the bunt fungi. Temperatures in the early stages of growth, according to Mouravieff (126), may help the plants to outgrow the fungus, but in the later periods of growth they play no part in determining the incidence of infection. In a study by Leukel (103), seed of Purplestraw wheat was inoculated with spores of *T. foetida* and germinated at 6°, 13° and 26° C. in soil in constant temperature tanks. At emergence one-third of the seedlings in each lot was retained at the initial soil temperature, while an equal portion was transferred to each of the other two temperatures. Finally, when the fourth-leaf stage was reached, all seedlings were transferred to a greenhouse bench to grow without further special control. Up to 97% of infected heads occurred in some lots, but none of the transfers from one temperature to another resulted in any striking difference in bunt.

Time of Seeding. The results of studies in various parts of the wheat-growing world are available on this phase of the bunt problem. Consecutive sowings of winter wheat in Kansas from September 15 to November 1, reported by Johnston (79), showed consistently more bunt than earlier sowings. After November 1, infection rapidly declined.

In Missouri, according to Reed (153), different dates of seeding winter wheats resulted in marked differences in smut (*Tilletia foetida*) in some years but not in other years. As noted by Reed, "soil moisture, soil temperature, and other possible soil conditions are interdependent factors which determine the occurrence of bunt in wheat. Their interaction determine whether infection will take place and also the severity of the attack. Any one of these factors may be a limiting one in the prevention of infection". In Australia, as noted by Neill (135), sowings made in the winter months, June, July and August, are more likely to yield a heavily bunted crop than earlier or later sowings. Using spring wheat and *Tilletia caries* in Siberia, Antonoff (7) made sowings at ten-day intervals in May. The later sowings invariably showed less smut. Also, in the later sowings, fewer seedlings were killed as a result of infection. Andreyeff (4), like Reed (153), concluded that the severity of infection with *T. foetida* depended on the whole complex of environmental conditions, including soil type, climatic and other factors. In a further study (5) he noted that later autumn sowings of wheat suffered more from bunt than earlier ones; also that bunt appeared to be more severe in areas where the rainfall was greatest during the vegetative period of the crop.

In Italy, according to Milan (118, 119), early fall and late spring seedings produced crops nearly free of *Tilletia caries* and *T. foetida* due to unfavorably high temperatures for infection at these times. Highest infections were obtained at air temperatures of 5.5° and 6.7° C. during seed germination. The results of Rabien (150) on influence of the date of seeding showed definite correlation between temperature and the degree of infection. In the trials with spring wheat, the temperature was 6.7° at the earliest date of sowing and 14.8° C. at the latest date. Every drop in temperature between the earliest and latest dates resulted in an increase in smut, while every rise in temperature resulted in a decrease in smut. In winter wheat tests late September and early October seedings developed more bunt than the November seedings.

The incidence of *Tilletia caries* in Knorrs' (92) studies was highest in late-sown winter wheats and early-sown spring wheats. The higher percentages of smut in spring wheat seeded March 23 over that sown April 6 were attributed by Knorr to the lower temperature of the earlier date, resulting in a prolongation of the period of susceptibility. Likewise in Russia, Abramoff (1) reported a progressive decrease of *T. caries* and *T. foetida* from the earliest to the latest plantings of spring wheat, and this also was attributed to the lower soil temperatures of the early seedings. In the United States the highest percentages of *T. caries* and *T. foetida,* in studies by Heald and Gaines (60), occurred in soil with a moderate moisture content in seedings made between the middle of September and early October at soil temperatures between 45° and 50° F. (7.2°–10.0° C.). Djelaloff (35), in tests in Azerbaijan, reported bunt percentages of 71.68, 44.0 and 15.7 in plants from seed sown on the first and fifteenth of November and the seventh of December, respectively. According to Arnaud and Gaudineau (11), the later seedings in France usually have more bunt than the earlier ones. Gaudineau (52) also reports making weekly sowings of Bon Fermier and Florence wheats from September 2, 1931 to the end of March 1932. The highest percentages of bunt were obtained in the spring sowings. Results presented by Mourashkinsky (124) in 1932 seemed to confirm the general view that infections of *T. caries* and *T. foetida* are less in late-sown than in early-sown wheat, but in a further study (125) late autumn sowing of spring wheats in western Siberia proved ineffective in reducing the attack of these fungi.

In other studies with spring wheat it has been reported that early sowings developed more bunt than later ones (61, 63, 127, 129, 130, 133, 173), or that no definite relation between sowing date and the incidence of bunt was found (128). In other studies with winter wheat, the most bunt appeared in the early sowings (127, 216) or in the intermediate sowings (9, 57, 63, 171), or no important differences occurred (111). Several investigations (89, 129, 130) are in accord that late sowing of winter wheat in autumn results in more bunt than early sowing the following spring.

Fertilizers. Fertilization with potassium and phosphoric acid increased bunt infection, according to Heuser (63), but nitrogen reduced it. Hiltner and Lang (65) reported pronounced effects of certain fertilizers upon the incidence of *Tilletia caries* and *T. foetida* in plants on two types of soil, one a heavy loam, the other a stony soil with an admixture of humus. The addition of potassium chloride resulted in an increase in infection on both soils. Smut was considerably reduced, however, by rhenania phosphate, 160 pounds per acre. Calcium cyanamid, 26 pounds per acre, reduced infection on the stony soil from 22.6 to 4.36% and on the loam from 13 to 10.7%. On both soils bunt was reduced to less than 1% by the addition of approximately 100 pounds of calcium cyanamid per acre. The best results were obtained when all three, potassium chloride, rhenania phosphate and calcium cyanamid, were added. Infection then was reduced to 0.52%.

At the Kharkoff Experiment Station in Russia it was conclusively shown, according to Buchheim (21), that in the early stages of growth of various cereals, the number of infected plants on manured plots is increased. Eventually, however, the percentage of smut is decreased due to the more vigorous development which enables the plants to outgrow the fungus. Buchheim did not specifically mention bunt, but it was probably included in the Kharkoff tests. The influence of fertilizers on *Tilletia caries* and *T. foetida,* according to Caspar (24), is not profound, but kainit is known to promote infection, while superphosphates, basic slag and calcium cyanamid impede it.

Straib (182) states that the degree of susceptibility to bunt fungi is an inherent varietal character that may be appreciably modified by fluctuating chemical, physical and climatic factors. He also reports trying calcium cyanamid, calcined lime and superphosphates as dusts on seed grain for bunt control (183). The results were considered hardly favorable enough to justify further attempts along this line in Germany. Rabien (150) studied the effects of fertilizers by applying the materials to soil in boxes. It was found, however, that the results, within the limits tested, were not reliable under actual field conditions. In some of the fertilized plots a correlation was found between abundant germination of the spores and high infection and between low germination of the spores and low infection, but in other cases no correlation could be found. Rabien concluded that, in general, the effects of fertilizers on infection of wheat by *Tilletia caries* is due not alone to their action on spore germination.

Feucht (42) studied several fertilizers and concluded that calcium cyanamid was the most effective in reducing bunt, although complete elimination of infection was not obtained. According to Mourashkinsky (124), sulphate of ammonia, superphosphate and a complete fertilizer had no effect on bunt, but it was slightly increased with potash. Ryzhkova (169) reported a decrease of bunt from 86% in the control plots to 35 and 63.3% in plots fertilized with superphosphate and sylvinite, respectively. The results of Ajroldi (2) showed that *Tilletia foetida* was increased with potassium chloride, decreased with ammonium sulphate and not affected by the application of superphosphate. Hanson and Tervet (56), however, found no correlation between the amount of bunt and the application of different rates of potash, ammonium phosphate, ammonium sulphate, cyanamid, acid phosphate and urea added separately to the soil.

In a different approach Sempio (177) grew seedlings in inoculated soil, then transferred them to nutrient solutions containing various substances, including alkaloids, glucosides, phenols and metals. After 20 days the seedlings were planted in soil. It was found at maturity that only cadmium, nickel and salicylic acid had any effect, the tendency being to reduce infection.

Soil Type. Faris (40) germinated seed inoculated with *Tilletia caries* and *T. foetida* in sand and potting soil at controlled temperatures favorable for infection. Two varieties of wheat were used. At the second leaf stage the seedlings were transplanted to a field. Only low percentages of bunt were obtained with *T. caries,* but up to 86.5% of the plants became smutted with *T. foetida*. In both varieties plants from seed germinated in the potting soil produced more smut than those from seed germinated in the sand, but the differences were not great. Striking differences were reported, however, by Gassner (50) in Germany. Four types of soil and the two species of bunt were studied. The highest infection (91.1%) occurred in sandy soil, followed by that on arable soil. Plants in moorland soil showed only a trace of smut, while those on clay were smut-free. In another test the smut percentages were 30.5, 29.2, 24.2 and 1.0 in plants grown in garden soil, sand, peat and clay, respectively.

In studies by Rapin (151) the bunt attack was much less severe in plants on sandy than on humus-rich soil. Leukel (103) found sandy soil less favorable to bunt than clay soil. Application of lime to acid sandy soil increased infection in plants from inoculated seed, indicating that acid soil inhibits infection. Walstedt (217) attributed an uneven distribution of bunt in an infected field to differences in the constitution of the soil, the clay-mould on a calcareous substratum of the upper part of the field being evidently more favorable than the deep clay of the lower portion.

In studies by Rodenhiser and Taylor (163, 164) it was found that a close relation exists between the degree to which a variety may become infected and the soil type and temperature during the period of subterranean growth of the wheat seedling from the seed to emergence above ground. As noted previously this is also the period of incubation and initial infection by the bunt fungi. When the seedlings emerged in Hempstead silt loam and Mendon loam, differences in bunt were obtained, but they were contingent upon the soil temperature during seedling emergence. At 10° and 15° C. there were no appreciable differences in amount of smut resulting from emergence in the Hempstead and Mendon loams. At 5° C., however, the Marquis variety developed 33% and Thatcher developed 42.2% more smut in Mendon than in the Hempstead loam. In another test consistently higher percentages of bunt developed in Chester loam than in Keyport clay loam when the incubation temperatures were 5° and 10° C. At 15° C., however, these differences were less except when the soils were highly acid; then 34.7% more smut developed in the Keyport than in the Chester loam. In the light of these results Rodenhiser and Taylor concluded: "Thus, soil type and temperature during the incubation period are closely interrelated in affecting the degree to which a variety may be infected".

Soil Zones. According to Russell (168) there appears to be a distinct correlation between the soil zone and the prevalence of bunt and other cereal smuts in Saskatchewan. Bunt was most prevalent in the brown soil zone and least prevalent in the black, while the reverse was true of the covered smuts of oats and barley.

Rate and Depth of Seeding. Caspar (24), Milan (119) and Mourashkinsky (124) reported more bunt in plants from heavy seedings than from thin seedings. Caspar also noted that bunt was less prevalent in well-loosened soil and when comparatively old seed was used.

In a comprehensive study of the effects of deep and shallow seeding on bunt, Woolman and Humphrey (222) sowed seed at six depths from one-half to three inches and obtained a direct correlation between increase in seeding depth and increase in bunt. In general, lowest smut percentages were obtained at the half-inch depth and the highest at the three-inch depth. There was usually little difference, however, in the results from the two-, two and a half- and three-inch depths. Caspar (24) and Fomin (44) likewise found that deep seeding promotes infection. Along similar lines Mourashkinsky (124) and Dobromysloff (36) reported that bunt was more prevalent in plants in ridged rows than in flat rows, and Jones *et al.* (83–85) in Egypt obtained five times as much bunt when the seed was plowed under as when it was harrowed in. According to Benloch (15) shallow seeding, seeding in dry soil or very early or very late seedings are conducive to a lowered incidence of bunt.

Vernalization. In studies of bunt as affected by vernalization or the sprouting of seed at low temperatures, Nemlienko (136) observed that the process was unfavorable to bunt, possibly because part of the spores applied to the seed had been killed in swelling and chilling. Evidence was obtained that infection takes place in the field after the seed has been sown and not during vernalization. In Lasser's (98) study the percentage of infection by *Tilletia caries* and *T. foetida* was reduced by vernalization, but the growth of winter wheat was not stimulated by this process.

Light. Several reports by Rodenhiser and Taylor (163, 165) show that length of day may have marked influence on the incidence of stinking smut in wheat. In one study (163) the variety Hope, exposed to light continuously, 10–11 hours daily and eight hours daily, developed 64.1, 17.5 and 0.8% smut, respectively. The corresponding percentages for the Marquis variety were 32.7, 17.8 and 1.9. In a further study by Rodenhiser and Taylor (165) Canus and Ulka wheats were inoculated with each of 3 physiologic races of *Tilletia foetida* and five of *T. caries.* Under natural-day conditions Canus was resistant to all races, but under continuous light (natural daylight supplemented with artificial light) a marked breakdown in resistance to certain races but not to others occurred. Ulka was completely susceptible to all but one race under both day-length conditions. Resistance to this one race was lowered by the long day treatment.

Mixed and Preceding Crops. Winter wheats with an admixture of rye had more bunt than pure crops according to Balakhonoff (13). Also the severity of bunt increased as the admixture of rye increased, although the rye remained free of bunt. Feucht (42) found that the preceding crop may influence the infection of wheat by *Tilletia caries* and *T. foetida.* Infection was higher following peas than following wheat. Mourashkinsky (124) noted that infection was highest on soil freshly broken from grass and alfalfa and lowest on soil that had been cultivated for several consecutive years.

Size of Endosperm. Westermeier's (219) results indicate that neither the size of the endosperm nor the state of nutrition of the embryo influences the degree of infection of either *Tilletia caries* or *T. foetida.*

Summary. Holton and Heald (67) present a valuable digest of factors that influence infection by *Tilletia caries* and *T. foetida* and their development in the host. It is as follows: "Temperatures ranging from 5° to 15° C. are highly favorable to infection while lower and higher temperatures are detrimental to infection. Soil moisture ranging from 15 to 60% of the water-holding capacity has been found to favor infection while infection is reduced in exceedingly dry or wet soils. The optimum soil moisture content appears to vary according to the soil type. Sandy soils and humus-rich soils favor infection while pure clay, peat, and highly acid soils are detrimental to infection. Fertilizers such as potassium, phosphoric acid, kainit and compost seem to stimulate the production of bunt while lime, nitrogen, superphosphate, calcium cyanamid and others are unfavorable. Up to a certain point (0.5 grams spores to 100 grams seed) the percentage of infection increases with increase in the spore load of the seed grain and the greater the concentration of the spore load around the embryonal zone the greater will be the degree of infection. It appears that bunt develops better in thick than in thin seedings made at a depth of 2 to 3 inches. There are indications that other factors influence the development of bunt but the present experimental evidence is scarcely adequate to justify general conclusions regarding these factors".

Head Smut of Corn and Sorghum (Sphacelotheca reiliana (Kuehn) Clint.)

Potter (147) conducted a three-year study to determine whether or not sorghum plants from seed produced in different localities might vary in their reaction to head smut. Plants were grown at Amarillo and Chillicothe, Texas, St. Paul, Minn., and Arlington, Va., from seed that was produced at each of these places. No evidence was obtained that indicated any measurable influence of the locality in which the seed was produced. In a study of soil moisture and temperature during seedling emergence on the incidence of head smut, Christensen (26) found that in a soil low in moisture (15% on an oven-dry basis) seedlings of sorghum became infected at temperatures from 16° to 36° C., the optimum being 28° C. In a moisture soil (25% on an oven-dry basis) the percentage of smutted plants was reduced at all temperatures, and the thermal range for infection was narrowed but the optimum remained at 28° C.

In a study along similar lines by Reed *et al.* (159), corn and Red Amber sorgo were inoculated with spores from corn and sorgo, respectively, by applying a day old spore-soil mixture to seedlings of the same age. In soil at 20, 40, 60 and 80% of its water-holding capacity, the percentages of infection were 50, 42.3, 71.4 and 51.8, respectively, for corn, 19.3, 3.5, 3.5 and 0.0 for sorgo. In soil at 20°, 25° and 30° C. (moisture content not given) the percentages of infection were 89.6, 77.2 and 84.6, respectively, for corn, 73.3, 29 and 18.9 for sorgo. Thus all three temperatures appeared to be equally favorable for the infection of corn, but a soil temperature of 20° C. during seedling emergence appeared more favorable than either 25° or 30° C. for the infection of sorgo.

Stalk Smut of Rye (Urocystis occulta (Wallr.) Rab.)

Stakman *et al.* (180) sowed inoculated rye seed in soil maintained at 14°, 19°, 24° and 29° C. during emergence of the seedlings. The percentages of infected plants were 47, 35, 24 and 0, respectively. In a study by Ling and Moore (108), controlled soil moisture and temperature conditions were applied from seeding of the inoculated seed to the two-leaf stage of the rye seedlings. The plants then were left to continue growth in a greenhouse without further special treatment. Under these conditions infection occurred at soil temperatures from 5° to 25° C. with decidedly less infection toward the two extremes of the range. Temperature optima for infection were 13° to 17° C. in the several tests. A soil moisture of 25% (air-dry basis) was considerably more favorable to a high incidence of smut than a 65% moisture, and the range of infection was narrowed in the latter.

In further thorough research by Ling (107) the influence of a considerable number of environmental factors on infection was studied, including the effect of soil reaction during seedling emergence on stalk smut in Dakold rye. Inoculated seed was sown, and seedlings emerged in the various lots of a soil adjusted to nine different reactions ranging from pH 5.49 to 11.92. Following emergence the seedlings were transplanted to a soil of pH 7.2, then left to grow without further special treatment. Infection reached the maximum (50% smutted plants) at pH 7.36, but good infections also were obtained at pH 5.49 to 8.58. Above this the decline in smut was rapid.

In an interesting experiment on the influence of soil moisture after seedling emergence, Ling (107) uniformly germinated inoculated Rosen rye seeds in soil at 25% of its water-holding capacity; then, beginning 10 days after sowing, the soil moisture was adjusted to 50 or 75% or continued at 25% and so maintained until the smut counts were taken. At the 25, 50 and 75% moistures the percentages of infected plants were 35, 29 and 14, respectively, in the first test, and 26, 24 and 19, respectively, in the second test. The results thus show that the development of *Urocystis occulta* in the host subsequent to the initial subterranean infection during seedling emergence is favored by a relatively dry soil.

Ling's (107) results with fertilizers indicated that solutions high in potassium phosphate and low in calcium nitrate and magnesium sulphate bring about a decrease in smut, while a reverse of these ratios tends to increase smut. Variation in intensity and length of light had little effect on smut in a spring rye, according to Ling (107), but lengthening the light period resulted in increased smut in Dakold, a winter variety.

Relative to seeding, Ling (107) found that with spring and winter ryes, seedings in Minnesota in late April and late September, respectively, were most favorable to smut and that shallow seeding at the one half-inch depth is less conducive to smut than sowing at depths of one to two inches. Also smut was somewhat higher in plants from seeds that germinated rapidly than in plants from slower germinating seeds. This held true for rye, however, in germination at 15° C., but not at 20° C. It also held true for wheat seed inoculated with *Tilletia foetida* when germinated at 10° C. but not at 15° C. Ling (107) also noted that in Dakold, and possibly other varieties of rye, smut-infected plants tend to be more easily killed during winter than smut-free plants.

Flag Smut of Wheat (Urocystis tritici Koern.)

Wheat flag smut, first found in the United States in 1918 (204) and first reported in 1919 (70), is now known to occur in Illinois, Missouri, Kansas and Washington (68).

In Australia, according to McAlpine (115), early sowing in a dry seed-bed following a dry summer is favorable to flag smut, since spores and seed germinate together when the rain comes. On the other hand, if the soil is moist and has been so for some weeks, most of the flag-smut spores appear to lose their effective power. Likewise, according to Verwoerd (213), field investigations in South Africa confirm the assertion of numerous farmers that flag smut is more serious in wheat sown before the first rain than in wheat sown after heavy rains. Noble (138) points out that he always obtained strong spore germination 12 to 18 hours after the addition of wheat plant tissue to spores which had been soaked approximately three days at 22°–24° C. in water. But spores which had been presoaked at lower temperatures and subsequently incubated at 27.5° C. rapidly lost their viability. The moisture-temperature relationship thus indicated is, according to Noble (138), possibly a factor in causing the smut reduction, frequently noted, when wheat is sown in infested soil sometime after the incidence of rain.

In a greenhouse experiment in South Africa, Verwoerd (213) sowed seed in inoculated soil in pots immediately after it had been moistened and one, two, three, four and eight weeks after the soil had been kept in the moistened state. The percentages of flag-smutted plants were 86.7, 75.0, 52.7, 38.6, 36.0 and 27.3, respectively. Relative to this decline in infection, Verwoerd (213) points out that, although some of the spores may have germinated in the moist soil and perished for want of host plants, all spores do not germinate quickly in moist soil. This was proved in a greenhouse test in which plants were grown from smut-free seed in each of four successive years in the same batch of heavily spore-charged soil in pots. Between crops the pots were protected against reinfection and set aside. Precaution against reinfection of the soil during the growing seasons also was taken by removing the plants as soon as the first symptoms of flag smut appeared. The percentages of infected plants in the first, second, third and fourth years were 86.7, 54.0, 33.2 and 9.7, respectively. This shows, according to Verwoerd (213), that soil moisture does not immediately cause all the spores to germinate or to lose their infectiousness.

In studies on soil temperature during seed germination, Griffiths (54) obtained percentages of infected plants as follows: 4.8 at 6°–12°, 8.5 at 17°–19°, 28.8 at 21.5°–23.5° and 0 at 25°–26.6° C. In a similar study Verwoerd (213) reported percentages of infected plants as 0 at 18°, 68 at 22°, 25 at 25° and 8.3 at 28° C. A soil temperature of approximately 22° C. (71.6° F.) during seed germination, therefore, seems highly favorable to infection. In a study on soil temperature effects by Noble (139), slightly sprouted seed was inoculated with dry presoaked or germinating spores. The seedlings were then sown in soil held continuously at controlled temperatures until the plants were well grown and had had ample time to develop smut sori if infected. Under these conditions soil temperatures ranging from 14° to 21° C. were optimum for infection. The most severe and earliest infection occurred on plants kept at 19° to 21° C. Some plants became infected at 5° and at 23° to 25° C. No infection occurred at 29° to 31° C. Inoculation at 15° C. and subsequent transfer of the plants to temperatures above 23° C. resulted in a decrease in the amount of infection. In a similar study by Faris (41) high infection was obtained at 10° to 20° C. when the soil was "rather dry", and at 10° to 15° C. when the moisture was at 40%, the point considered ideal for sowing wheat. When the soil was almost saturated (60%) high infection was obtained only at 10° C., and percentages of smut dropped very rapidly at temperatures lower and higher than this.

Tisdale *et al.* (203) made a three-year study of infection in the susceptible Harvest Queen wheat as influenced by the date of seeding at Granite City, Illinois. Wheat sown early in October smutted more than wheat sown later in the fall. There was little difference, however, in the amount of smut in sowings made sufficiently early to insure a good crop of grain. Sowings made after the first of November showed much less smut than earlier sowings. Sowings made after the middle of November were smut-free, but the yields were very low. In a study along similar lines at St.

Louis, Mo., Griffiths (54) sowed inoculated seed of Harvest Queen winter wheat at three-day or four-day intervals from October 17 to November 21, 1922. The three highest percentages of infected plants, 61.3, 50.0 and 37.8, occurred, respectively, in the first three seedings on October 17, 20, and 24. The three last seedings, November 14, 17 and 21, were smut-free. From 14.0 to 29.2% smutted plants occurred in the intermediate seedings. In a recent report on flag smut in China by Yu *et al.* (223) it is noted that the amount of smut produced depends largely on the soil moisture and temperature during seed germination and that the time of sowing therefore is highly important. At Kunming the optimum conditions occur at the time for sowing wheat, or a little before, when the soil temperatures range from 13° to 25° C. Sowing wheat in the latter part of November usually results in low infections due to the low soil moisture at that time.

A report by Carne (23) indicates that flag smut is promoted by deep seeding, heavy rather than light soils, and dry seeding rather than wet seeding. It is also likely to be serious in self-sown crops.

Miller and Millikan (120) in Australia found that soil conditions favorable to infection are temperatures above 50° F., lack of excessive moisture and a pH range of 5.5 to 8.7. Deep seeding and other conditions tending to prolong the subterranean period of the seedling also promoted infection.

According to Forster and Vasey (45), lime up to 10 cwt. per acre increases flag smut. Farmyard manure also results in increased smut. When both are applied, infection is particularly severe. No appreciable effect from superphosphate has been observed.

In studies by Millikan (121) with the susceptible Free Gallipoli wheat, an excess of calcium increased flag smut, while a deficiency of this element practically inhibited the disease. With the resistant Ghurka variety excess calcium again resulted in an increased infection, but significant results with a calcium deficiency were not obtained. In further studies by Millikan (122) a calcium excess of twice the normal dosage significantly increased flag smut in the Free Gallipoli plants, but a four times normal excess had no effect. With the resistant Ghurka no treatment increased susceptibility to any degree comparable with that of the susceptible controls grown under the same conditions.

Millet Smut (Ustilago crameri Koern.) and Broomcorn Millet Smut (Sphacelotheca destruens (Schlecht.) Stevenson and A. G. Johnson)

Two remaining seedling-infecting cereal smuts are millet smut (*Ustilago crameri*) and broomcorn millet smut (*Sphacelotheca destruens*). Apparently no studies dealing with their environmental aspects have been reported.

THE SHOOT-INFECTING CEREAL SMUT

In this type of infection, illustrated by the common corn smut, the fungus attack is not systemic but localized to various aerial parts, stalks, leaves, ears, tassels and even brace roots. Here the fungus produces fleshy galls of various sizes, which become filled with immense numbers of dark brown chlamydospores. These spores are scattered over the corn field in late summer and autumn through drying and disintegration of the smut galls, and through spreading manure from stock fed with smutted fodder. The following spring, under appropriate temperature and moisture conditions, chlamydospores that have survived the winter, germinate and produce secondary spores or sporidia that are blown to the corn plants and initiate the localized infections. Sporidia fall into the leaf whorl of the growing corn plants, and so into the water from rain, dew or guttation within the leaf sheaths about the tender tissues of the growing points at the nodes as well as in the young tassel. The fungus also may infect the silks and through them gain entrance to individual young kernels on the ear enclosed by the husks. Each smut gall that eventually appears represents a separate infection.

Corn Smut (Ustilago maydis (DC.) Cda.)

In view of the life history of the corn smut pathogen noted above, high atmospheric moisture would seem essential to its development. Fifty years ago, however, Hitchcock and Norton (66) stated: "Sofar as our observations go, smut is more abundant in dry seasons, and in the drier localities". A year later Selby and Hickman (175) expressed a similar opinion. Likewise Potter and Melchers (148) have reported that corn smut is more prevalent in dry than in humid seasons and regions. They doubt that moisture can be a factor in smut development under climatic conditions such as corn requires. In semi-arid regions they frequently found 60 to

80% of the stalks affected. Reporting on smut in selfed-line nurseries frequently sprayed with spore suspensions, Immer and Christensen (74) observed that dry weather conditions, as expressed by a low number of days of precipitation and a high percentage of sunshine, were conducive to smut. Temperature was not so important a factor as either number of days of precipitation or percentage of sunshine. In studies by Platz (145) a survey of corn smut in 1923 and 1927 near Ames, Iowa, showed that smut was more prevalent in 1927. In the latter year the mean temperature during the growing season was somewhat lower than in 1923. Also the rainfall during June, July and August in 1927 (3.92 inches) was much less than that for the same period in 1923 (13.79 inches). A season in which relatively cool dry weather in June was followed by a period of rain and higher temperature seemed to favor the disease. Arthur and Stuart (12) concluded that infection takes place during cloudy days and dewy nights. They note, however, that in especially wet seasons less smut usually occurs than in dry seasons. In studies by Piemeisel (144) cool damp weather favored infection, and thick planting with consequent shading accounted for the greater prevalence of smut in silo corn than in field corn.

In greenhouse tests Platz (145) found that high relative humidity favors infections. Also under greenhouse conditions Tisdale and Johnston (205) reported that the incidence of smut is increased by high temperatures, 80° to 95° F., and by sufficient moisture to prevent drying of fluid inoculum until infection takes place.

In observations from 1917 through 1923 at Akron, Colorado, Coffman *et al.* (29) determined that scant precipitation in May and June followed by moderate rainfall during July and August results in the highest percentages of infection. Rainfall distributed throughout the season promotes only moderate infection, while heavy rainfall during the early part of the season followed by dry weather the latter part of the growing season results in low infection. Their explanation of these findings is that early season rainfall induces germination of the chlamydospores and exhaustion of the supply of inoculum before the corn is large enough to be infected. Dry weather in May and June, however, keeps the chlamydospores viable so that rains in July and August bring about a maximum supply of sporidia at the time when the corn is most susceptible.

Even a few light rains in July and August are sufficient to provide the moisture necessary for chlamydospore germination, sporidial production and the multiplication of sporidia in the infection courts of the host. From observations in Iowa, Platz (145) reached similar conclusions.

Piemeisel (144) reported the interesting discovery that the sporidia long believed to be easily killed by drying may still be viable five months after drying.

Relative to temperature Jones (81) has shown that both chlamydospore germination and sporidial production require comparatively high levels, the optimum for both lying between 26° and 34° C. She also found that the chlamydospores do not germinate readily on many soils. This may be tied in with soil reaction, since Platz *et al.* (146) have reported that a pH of approximately 4.9 is optimum for germination. According to Tisdale and Johnston (205), the usual freedom of very young corn plants from smut galls is due to low temperature rather than age. They discovered, moreover, that the seedlings of smut-resistant varieties are often rather susceptible but become resistant as the plants grow older.

Kyle's (96) work on protection by the husks of corn ears against smut is an interesting example of the ramifications that may emanate from environmental influences. He found that lines of corn with ears well protected with husks had less smut than lines not so well protected and that the relative sizes of ears and husks varied with environment, causing fluctuation in the quality of protection. Kyle (97) also reported that increased susceptibility to smut is an accompaniment of increased plant vigor, whether due to varietal or hybrid vigor or to soil fertility, soil moisture, cultivation, *etc.* According to Walter (218) sustained rapid development of plants to full stalk size in many cases favors escape from or resistance to smut, but a direct relationship between vigor of the host and susceptibility to smut was noted only for plants of extremely low vigor and certain weak selfed lines.

Vohl (214) reported that the incidence of smut rose with increasing applications of nitrogen fertilizer, averages of 50.8, 53.1 and 61.3 smutted plants being counted per sq. m. on plots receiving the equivalent or 40, 60 and 80 kg. of pure nitrogen per hectare, respectively. A similar relationship was observed in a later test when the smut percentages were 5.1, 6.2, 6.9 and 8.9 in plots receiving 0, 40, 60 and 80 kg. nitrogen per hectare, respectively.

THE FLORAL-INFECTING CEREAL SMUTS

This group includes only the loose smut of wheat (*Ustilago tritici* (Pers.) Rostr.) and the deep-borne loose smut of barley (*U. nuda* (Jens.) Rostr.). In both, as with the other small grain loose smuts, *U. avenae* and *U. nigra,* the smutted heads appear at heading time and begin shedding their spores during the bloom period of the normal heads. However, spores of *U. tritici* and *U. nuda* that come in contact with wheat and barley flowers, respectively, germinate by forming no sporidia but only threads that grow down the stigma or through the ovary wall into the developing seed. Thus they produce an internal infection of the seed rather than an infestation of its superficial layers, as with the seedling-infecting loose smuts of oats and barley. As shown by Freeman and Johnson (46), moreover, after the ovary has attained more than one-third of its mature size, initial infection rarely occurs, and, as noted by Maddox (110) in his pioneering studies of floral infection, "The loose smut germs on the grain or mixed with the soil are harmless and will not reproduce the disease".

The loose smut of rye also should be included in this group, as Humphrey and Tapke (71) have shown that it also is caused by *Ustilago tritici.*

Loose Smut of Wheat (Ustilago tritici (Pers.) Rostr.) and the "nuda" Loose Smut of Barley (U. nuda (Jens.) Rostr.)

These two smuts may be considered together, since they are practically identical except that *Ustilago tritici* attacks wheat and rye (71), while *U. nuda* attacks barley. Apparently no comprehensive studies have been made to determine the ranges of temperature and humidity favorable for the floral-infection of wheat and barley. Doubtless this is largely due to the difficulties in constructing chambers that afford light in addition to control of humidity and temperature. It has long been observed, however, that these and the other small grain loose smut usually are scarce in sections of the country where the humidity of the air is low at flowering time. Mackie (109) notes, for example, that in California the dry season is near at hand when cereal plants are heading and that the damage from loose smuts in wheat, barley and oats in that State is too slight to be of economic importance. In a study of humidity in relation to the incidence of loose smut in wheat, Tapke (187) used three club wheats, Hybrid 128, Jenkins and Little Club that are grown on the west coast and rarely develop loose smut there. However, when grown from seed from flowers that had been inoculated by hand under humid conditions in a greenhouse at Arlington, Va., these varieties proved extremely susceptible, 90 to 98% of the plants being severely smutted in heads and leaves. Flowers of Federation wheat and Multan and Hanna barley also were inoculated on plants growing on irrigated land or in large cans that were taken to a desert at flowering time. Inoculations under the relatively humid conditions of irrigation resulted in 62 to 87% of smutted plants, while those in the dry air of the desert resulted in 0 to 18% of smutted plants. In still another test, heads of Little Club wheat were florally inoculated by hand, then left exposed either to the air of a greenhouse at 56 to 85% relative humidity or the heads were inserted in a chamber with air reduced to 11 to 30% relative humidity through the use of calcium chloride. Seed from the flowers inoculated under the moist and dry conditions produced plants with 94 and 22% of smutted heads, respectively. Daily microscopic examinations of inoculated flowers showed that under humid conditions, spores on the stigmas soon developed long germ tubes, whereas under the arid conditions spores germinated much more slowly. The results of the studies leave no doubt that low atmospheric moisture at flowering time is inimical to high infection, probably because spores enclosed within the glumes germinate too slowly to reach the ovary during the short period during which it is vulnerable.

An interesting account of the influence of air currents on the dissemination of spores of the wheat loose smut fungus (*Ustilago tritici*) has been contributed by Oort (142). His results from two experiments in Holland show that the spores may be disseminated and cause infection at least up to 100 meters from the point of origin. In winds of high velocity the dissemination of spores occurred in a horizontal plane at the level of the healthy heads. The infection was heavy but diminished regularly with distance from the source of inoculum. In winds of low velocity infection, in general, was much less and it was spotty. Islands of infected areas alternated with those of smut-free areas. These were a reflection of the influence of vertical air currents that picked up inoculum, then dropped it in spots, as has been observed in the spread of rye pollen during flowering time in a weak wind.

According to Tschermak (207) under the conditions of a moist warm atmosphere and a dry soil, flowering proceeds rapidly while the head is still in the boot, thus minimizing the chances of floral inoculation. On the other hand, rain followed by sunshine causes the heads to emerge before blooming and the glumes to spread widely, thus favoring the chances of floral inoculation. Cool weather also causes a longer flowering period outside the boot and likewise favors the entrance of spores into the flowers. According to Leighty and Sando (99) nearly 5% of the wheat flowers observed in their study bloomed without opening. They note also that under certain environmental conditions wheat flowers may be rather generally cleistogamous. It is, therefore, evident that weather conditions at flowering time are vital factors in the incidence of *Ustilago tritici* and *U. nuda* through their influence on exposure of the flowers, dissemination of loose smut spores and germination of the spores after they get into the flowers.

In addition to the atmospheric influences noted above, the incidence of loose smut in wheat and barley also may be affected by various conditions of the soil. It will be apparent, however, that much of the information on this phase is not clear cut. Concerning soil temperature effects during seedling emergence on *Ustilago nuda,* Leukel (102) germinated seed of Alpha barley from hand-inoculated flowers in soil at 5°, 10°, 15°, 20°, 25° and 30° C. After emergence all plants were uniformly grown under greenhouse conditions. From 44.4 to 56.3% of the plants of the different lots became smutted, indicating little if any significant reaction to wide differences in soil temperature during seedling emergence. Neill (134) likewise found that different preemergence soil temperatures had little effect on the incidence of *U. tritici.* Tieman (202) states, however, that a late seeding of spring wheat at relatively high temperature increased *U. tritici;* and Gassner and Kirchhoff (51) noted a decline in *U. tritici* and *U. nuda* resulting from low germination temperatures. According to Hori (69) early seedings of winter wheats and barleys in Japan show high amounts of loose smut, while later seedings show less. This explains why these grains show little smut when they follow rice. The rice harvest delays sowing a month and a half or more. Oort (143) also found that a warm soil promotes loose smut in wheat and barley. On this account he sows winter varieties earlier and spring varieties later than usual in order to bring the loose smuts to their fullest expression in tests of varietal resistance. Freeman and Johnson (46) report that the time of planting spring wheat and barley had no effect on loose smut in Minnesota but with Tennessee Winter barley in Texas, interesting results were obtained. The crop developed 10–12%, 22–25% and less than 1% when sown September 21, October 12 and November 11, respectively. Since October 12 is the usual seeding time, the data suggest that this might be also the optimum period for smut development. In a three-year test by Sidorin *et al.* (178) a spring wheat sown in late autumn in the region of Moscow had no loose smut, but when sown in spring, 2.1 to 3.2% appeared.

Information on the incidence of *Ustilago tritici* and *U. nuda* as affected by fertilizers and soil fertility indicates that the influence of these factors probably is without practical significance in control. Some investigators have reported more loose smut in wheat or barley or both on fertilized plots (65, 106), but others have reported more on unfertilized plots (47, 51) or that fertilizing resulted in no appreciable change (202).

According to Seiffert (174) a reduction of the seedlings' period of subterranean environment through shallow seeding appears to minimize the tendency of barley to become infected by *Ustilago nuda.* Taylor and Zehner (197) reported that loose smut in Tennessee winter barley was from 2.1 to 44.5 times greater in amount in the three-inch seeding as compared with the half inch depth. However, in the winter varieties Esaw and Beardless No. 6 no significant difference in loose smut resulted from variation in seeding depth. These studies were conducted before the second loose smut of barley, caused by *Ustilago nigra,* had been discovered (188); and as noted by Tapke (189), there is reason to believe that Taylor and Zehner were dealing with *U. nigra* on Tennessee Winter and with *U. nuda* on Esaw and Beardless No. 6. If this assumption is correct then depth of seeding had no effect on the incidence of *U. nuda* in those studies.

In recent years a few reports have dealt with the effects of vernalizing winter wheats and barleys on *Ustilago tritici* and *U. nuda.* According to Hanna (55) and Bever (16) this process has little or no effect on the incidence of *U. tritici* in plants from seed from artificially inoculated flowers. Data cited by Zuhr (227), however, show that vernalization appreciably reduced bunt and loose smut in a spring variety and in a facultative variety of wheat grown in the field. Lasser (98) reported that vernalization induced winter wheats and barleys to produce heads in the greenhouse, but the process so greatly reduced infection of the wheat and barley seedlings with bunt and loose smut, respectively, in artificial inoculation tests, that studies in breeding for resistance were rendered useless. In a report on the effects of soaking and freezing wheat seeds before sowing, Buchheim (22) states that seeds infected with *U. tritici* survived the soaking and freezing as well as non-infected seeds and that plants from infected seeds thus treated produced nearly as much smut as those from infected seeds not treated.

In studies by Tapke (186) more winterkilling occurred in wheat plants grown from infected seed under the winter conditions at Ithaca, N. Y., than under the milder conditions at Rosslyn, Va.

ADDITIONAL CEREAL SMUTS

Leaf Smut of Rice (Entyloma oryzae (H. and P. Syd.) and Kernel Smut of Rice (Tilletia horrida Tak.))

Rice kernel smut (*Tilletia horrida*), according to Anderson (3), appears to be a seedling-infecting fungus, but, as noted by Fulton (48), the evidence is open to question. A recent report by Chowdhury (25) indicates that a direct infection of the kernels takes place as a result of floral inoculation by sporidia during anthesis or in the dough stage of seed development. It has been shown in recent years, moreover, that the chlamydospores will germinate and produce sporidia under certain environmental conditions (105, 198). According to Reyes (161, 162) rice grown in the Philippines under irrigation in the dry season seems more susceptible than that grown during the wet season without irrigation. Apparently no further studies on the environmental aspects of rice kernel smut have been reported. As noted by Tullis (211) usually the disease is of minor importance in the United States.

Relative to the leaf smut of rice (*Entyloma oryzae*) little definite information seems available concerning its life cycle and method of infection. Apparently no reports on the influence of environment are available.

CONCLUSION

It was noted in the introduction that E. J. Butler is credited with the statement that the present period in phytopathology is one in which disease is considered as an interaction of the host and the parasite "under the conditioning influence of environment". The foregoing pages lend ample proof of the truth of that statement.

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SOME ANATOMICAL ASPECTS OF PLANT VIRUS DISEASE PROBLEMS. II[1]

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INTRODUCTION

The present review compiles the literature of the past decade on the anatomic effects of plant viruses and correlates the old and the new information on the nature of the internal symptoms and their bearing upon the subject of plant tissue relations of viruses. The studies on the anatomy of plants affected by viruses continue to occupy a relatively small space in the literature on virus diseases. The lack of greater interest in the anatomic aspects of these diseases is regrettable because the knowledge of structural changes induced by viruses in plants contributes much toward the understanding of the nature and behavior of viruses. The anatomic approach is, for example, of the utmost importance for the determination of the relation of viruses to plant tissues, and by elucidating this relationship assists in classifying viruses. Anatomic studies on symptom development often make possible a distinction between primary and secondary symptoms and thus reveal some of the causal relationships in the development of the external symptoms. Comparisons of the anatomic effects of viruses with those induced by other agencies could clarify the nature of virus injury. Furthermore, these virus effects may prove of diagnostic value, especially if viruses are otherwise difficult to distinguish. Finally, ontogenetic studies on the malformations and other abnormalities induced by viruses are of interest with regard to plant morphogenesis in general, since normal phenomena are often elucidated through work on abnormal growth and differentiation.

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THE NATURE OF ANATOMICAL MODIFICATIONS INDUCED IN PLANTS BY VIRUS DISEASES

The current literature on virus diseases continues to mention the same fundamental types of pathologic alterations as the older: hypoplastic, hyperplastic and hypertrophic changes in the mesophyll and other parenchyma; depressive or destructive effects upon the chloroplasts; enations, some leaf-like; necrosis in various plant parts and tissues; degenerative changes in the phloem, sometimes expressed principally as sieve-tube necrosis, sometimes as growth anomalies followed by necrosis. An unusual type of abnormal growth resulting in the production of tumors has been described (15).

The effects of viruses on woody plants are receiving more and more attention—a trend, associated with the increase of interest in virus diseases of shrubs and trees (25); and since many woody species affected by viruses show disturbances in the xylem, the alterations in this tissue are being studied. Usually the xylem appears to respond to virus infection with a gummous degeneration. The intracellular rods (possibly trabeculae), previously described in tracheary elements of virus-diseased plants, have now been interpreted as structures not connected with the diseases (4).

As in the past, authors generally consider it characteristic that the most pronounced symptoms occur on young organs, which are still differentiating when the virus invades the plant. Considerable evidence has accumulated, however, that old organs may develop conspicuous symptoms. (See part on tissue relations of viruses.)

Some symptoms induced by viruses—internal as well as external—are more specific than others, but probably none is sufficiently specific that it cannot be reproduced by one or another agency besides a virus. Particularly striking is the resemblance of many virus-disease symptoms to the changes obtained in plants by the application of growth-promoting substances; and it is worthy of special emphasis that growth substances, like viruses, not only produce local effects but also have formative influences lasting for a considerable time. Zimmerman (142) even speaks of "systemic effects" in relation to such formative influences. Of course, the effects of viruses persist longer than those of the externally applied hormone-like compounds because, unlike the latter, viruses multiply in their hosts. At the same time, Kunkel's (81) successful experiments on curing virus diseases by heat treatments suggest that the effects of the viruses, as those of the growth substances, last only as long as these agencies are allowed to act upon the plant.

The effects of viruses have also been compared to those resulting from injuries or derangements of genes (112). Some viruses induce morphologic and physiologic changes without apparent necroses, and such irregularities usually belong to the group of teratological phenomena, either of a suppressive or of an activating kind. Of course, the exact nature of virus action, as well as of that of the genes, is not known, but both may have something to do with the function of the growth-substance mechanism in the plant.

Experimental evidence indicating an interrelation between viruses and the growth-substance activities in the plant is available in the literature. Jahnel (65, 66) reported that virus-infected potato plants contained less growth substance and reacted less strongly to an application of such substances than the healthy plants.

The significance of the similarity between the effects of viruses and those induced by many other agencies has been elucidated by some researches on the manner in which viruses bring about morphologic and physiologic changes in the hosts. According to McKinney and Hills (91), relatively small amounts of virus may induce symptoms in remote zones by translocated or diffused products of a deranged metabolism. The authors introduced a tobacco-mosaic virus into a small area of a leaf of perennial pepper and found that the inoculated leaf developed chlorosis in parts that contained no virus and that the systemic mottling in uninoculated leaves, too, occurred in the absence of virus from these leaves. A possible relation of symptom development to a changed enzyme balance was suggested by some biochemical tests (91). In tobacco the authors found no mosaic mottling before the arrival of the virus in the mottled parts, but decided that this observation did not preclude the possibility that the mosaic symptoms in tobacco, as well as in the pepper, were induced directly by products of a deranged metabolism.

The long-distance effect of viruses upon the metabolism of the plant was brought out also by the researches of Wynd (135–137) who observed that the uninoculated plant parts showed alterations in enzymatic activities and in the use of oxygen several days before the virus appeared in infectious concentrations in these parts.

The evidence that symptoms of virus diseased plants may be expressions of a disturbed metabolism agrees with the common observations that the type of symptom which eventually develops after infection is affected by the age of plant or organ (*e.g.*, 90), by varietal differences, and by environmental conditions (*e.g.*, 19). It is yet to be reconciled, however, with the reports of a very specific relation of viruses to symptom expression. Caldwell and James (19), for example, concluded that the hypertrophy, hyperplasia and inhibition or destruction of chlorophyll in the palisade cells of narcissus affected with the stripe disease were entirely separate phenomena, each probably induced by a distinct component of a virus complex. In view of the available information that the combination of cell enlargement, cell division and destruction of chlorophyll is very common and occurs in plants affected by various injurious agents (82; see also 31), Caldwell and James (19) would have to provide strong evidence to prove the existence of the specific virus action postulated by them. Such evidence is apparently not yet available with reference to the stripe disease of narcissus (20). Smith (119), however, reports having separated two components, one inducing mottling and the other a distortion of veins, in tobacco rosette disease. One component virus was found to be transmissible by aphides, the other by the same aphides and by mechanical means. Mottling and vein distortion often occur as distinct symptoms induced by different viruses. Evidently Smith is dealing with similarly distinct viruses that differ in their effect upon the metabolism and induce unlike symptoms.

Symptoms in Leaf Parenchyma. The most frequently studied symptoms in leaf parenchyma are those induced by mosaics. Like the papers previously reviewed (33), the new studies dealing with the anatomical effects of mosaics continue to describe hypoplasia in the light-green or yellow areas of diseased leaves, underdevelopment of plastids and sometimes also a destruction of the latter (*e.g.*, 24, 38, 121). Occasional papers, some dealing with mosaics, others with other types of virus diseases, report somewhat different combinations of pathologic alterations. According to Caldwell and James (19), in the stripe disease of narcissus a deficiency in chloroplasts is combined not with hypoplasia but with a hypertrophy and hyperplasia of palisade cells which often push through the epidermis and form ridges on the surface of the leaf. An association of hypertrophy of mesophyll cells and chloroplast destruction occurs also in the bulging parts of beet leaves affected with virus yellows (77). The yellow streaks in sugar cane affected with the chlorotic streak show reduction in size and number of chloroplasts, are often thinner than the adjacent areas and eventually become necrotic (1). In contrast to the just mentioned diseases, the bunchy top of banana apparently causes an increase in the number of chloroplasts, namely, in the parenchyma along the veins (87). This parenchyma becomes subdivided into small-celled tissue rich in chloroplasts and gives the effect of dark-green streaks when the leaves are viewed from the surface.

Considering together the literature reviewed previously (33) and the recent papers, the results of studies on diseases primarily affecting the mesophyll, that is, mosaic types of diseases, may be conveniently summed up as follows. The degree of difference between the yellow and the green areas, on the one hand, and between the healthy and diseased leaves, on the other, vary considerably. Similarly the profoundness of the cytologic effects of viruses, such as the abnormal starch accumulation, or the inhibition of plastid development, or the occurrence of chloroplast destruction, varies much in degree. Viruses naturally differ in virulence, and furthermore, the type and severity of symptoms is affected by the age of the leaf at the time of infection. Thus Stone (121) found little effect of the mild mosaic virus upon the leaf thickness in potato, whereas Hoggan (57), using other mosaic viruses, observed the usual reduction in thickness of the yellow leaf areas on solanaceous hosts. Goldstein's (46) study, on the other hand, showed that leaves infected when still rather young exhibited more marked internal abnormalities than those that were older when the virus entered them. Various environmental conditions also affect the expression of symptoms, as is evidenced particularly well by the masking of symptoms under certain conditions of light and temperature in many mosaics; and the differences in starch accumulation must be judged in the light of the observations that viruses delay both accumulation and removal of chloroplast starch. The susceptibility of symptom expression to various internal and external factors might well explain the conflicting data in the literature on the anatomic and cytologic abnormalities in mosaic diseases.

The hypoplasia in the yellow areas of mosaic leaves merits further attention from students of pathological anatomy. In partly or fully developed leaves the yellow mesophyll usually appears more juvenile than the green. Certain developmental studies indicate, however, that the yellow areas do not always appear less differentiated than the green parts. In the early stages of development of mosaic leaves, cell division is suppressed in the yellow areas (23, 29, 38, 64). Furthermore, according to Iwanowski (64), the yellow areas precociously develop intercellular spaces, so that in young leaves these parts seem to consist of a tissue older than the still dividing, compact green mesophyll. Then, Grainger and Heafford (47) reported that certain groups of mesophyll cells in leaf primordia of mosaic tobacco underwent early vacuolation and suggested that these cell groups would become the yellow areas in the mature leaf. The suppression of cell division, the appearance of intercellular spaces and the precocious vacuolation of cells indicate that the yellow leaf areas complete their differentiation before the green parts. Apparently, then, in the early stages of differentiation the yellow areas are hyperplastic (in the sense of Küster, 82), since certain phenomena of maturation occur in them earlier than elsewhere in the mesophyll; later, however, they appear hypoplastic because they do not complete the typical differentiation of mesophyll tissue.

As mentioned in the previous review (33), some workers attribute the abnormalities of the yellow areas of mosaic leaves to underdevelopment only; others describe destructive changes in the affected protoplasts as well. The proponents of the view that underdevelopment causes the scarcity and small size of chloroplasts have given good evidence to support their concept. At the same time, convincing data are available to prove that more or less fully developed chloroplasts also are affected by mosaic and other viruses. The present writer (38) has observed that though the yellow areas of beet leaves affected with mosaic showed chloroplast deficiency in very early stages of development, a degeneration of chloroplasts also occurred in older leaves. The chloroplasts were pale and fragile and they responded with swelling and disintegration to contact with tap water more readily than normal chloroplasts; or the chloroplasts were diffuse and irregular in shape, and stained lightly. In the most severely affected cells the chloroplasts fused into amorphous masses. The review of literature given in connection with the study on beet mosaic (38) shows that similar degenerative changes as in this disease have been recorded in other mosaics, but most often in connection with effects of injurious agents other than viruses—an example of lack of specificity in the action of the virus.

A good proof of the occurrence of destructive changes induced by viruses in differentiated chloroplasts is given in the observations that yellowing is sometimes observed in the more or less mature leaves. Rather old inoculated leaves may develop a considerably extended chlorosis, in addition to the local lesions (119, 127); and when in its systemic spread the virus is able to enter full grown leaves, these too develop prominent symptoms (55, 127). A cytologic evidence of plastid destruction by virus infection of grown leaves was found in the present writer's study on beet mosaic (38): the yellow spots that developed at the site of inoculation and the yellow strips of mesophyll that were located along the larger veins, through which the virus moved out from the place of inoculation, showed small or diffuse or fragmented chloroplasts, or the entire protoplasts were replaced by flocculent material. As Bawden (6) has suggested, the common absence of symptoms in leaves that are mature when the plants are infected is probably explained by the low virus content of such leaves, rather than by the inability of the virus to affect mature plastids. Thus it appears that underdevelopment and actual destruction of plastids play a rôle in the differentiation of the chlorotic patterns in virus-diseased plants.

In connection with the chlorosis of virus-infected leaves, the alterations that occur in the mesophyll along the veins deserve special mention because the terminology regarding these alterations is somewhat confused. Certain diseases, *e.g.*, the curly top of beet, bring about a translucency[2] of the small veins on the young leaves; that is, the veinlets of such leaves permit the passage of the light more readily than those in healthy leaves. Other diseases, for example, the Hyoscyamus III disease (113), or the potato Y-virus disease (6, p. 16), cause a yellowing of the leaf areas immediately aligning the veins. Translucency and yellow discoloration along the veins may be combined in the same leaves injured by viruses or by saliva of insects (109–111). Some workers (*e.g.*, 6, 113) apply the term "vein clearing" to both of these vein symptoms; others (*e.g.*,

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[2] This symptom is called "transparent venation" in some of the literature on curly-top, but its substitution by "translucent veins" was recommended by Esau (31).

109–111) use "clearing" in the sense of "becoming translucent" and employ "vein banding" when yellowing occurs along the veins. Writers who speak of "clearing" when the veins are outlined by yellow mesophyll (6, 113) reserve "vein banding" to characterize the appearance of dark-green tissue along the veins. The terminology regarding the alterations of leaf blades along the veins is obviously in need of stabilization. It would seem that the use of "clearing" only in the sense of "becoming translucent", and "vein banding" in the sense of differential coloring, either yellow, white or green, along the veins would be most appropriate.

How the phenomena of translucency and yellow vein banding compare with each other histologically and cytologically is not yet clear. The translucency of the vein in curly-top beet leaves depends on hyperplastic changes in the parenchyma associated with these veins: cell enlargement and cell division bring about a close packing of parenchyma cells on the phloem side of the bundle, and these growth changes are accompanied by chloroplast degeneration (31). The translucency of veins is the early expression of growth aberrations that later lead to a thickening of veins and a formation of wart-like enations characteristic of curly-top diseased leaves. Though the hyperplasia in the phloem is the primary symptom in curly-top infection, and the hyperplastic changes in the adjacent parenchyma are obviously secondary (31), the latter sufficiently explain the phenomenon of vein translucency: a tissue lacking intercellular spaces and deficient in chlorophyll would be expected to transmit more light than the normal lacunose mesophyll. Similarly, the larger veins of beet leaves are normally somewhat translucent because their vascular bundles are imbedded in large-celled parenchyma, a tissue less lacunose than the mesophyll and with relatively few chloroplasts. Growth changes resulting in the substitution of normal chlorophyll-containing parenchyma and of the mechanical sheath by large cells deficient in chlorophyll is responsible for the translucency of the large veins in *Musa textilis* affected with bunchy top of banana (87). Other examples of growth anomalies producing translucent tissues are available in the literature (31). They all indicate that enlargement and division of cells not accompanied by development of a prominent intercellular-space system but with a deficiency of coloring matter give the character of translucency. On the contrary, the presence of abundant intercellular spaces, as in certain proliferations associated with the stripe disease of narcissus (19), may make the tissue silver-gray in appearance.

The yellow vein banding has apparently been studied histologically and cytologically only by Sheffield (113) in reference to Hyoscyamus III disease in tobacco. She found no anatomical nor cytological abnormalities in the yellow areas, but a retardation of chlorophyll formation. The yellow-vein banding stage in Hyoscyamus III disease ("vein clearing" in Sheffield's terminology) is followed by a thickening—through a hypertrophy of the palisade cells—and a development of deeply green coloration of the formerly yellow areas (vein-banding stage, according to Sheffield). The possibility that yellowing of a tissue, like translucency, could be a sign of incipient hypertrophy and hyperplasia is also suggested by Caldwell and James's (19) observations on the stripe disease of narcissus. However, the yellowing of mesophyll in the mosaics is usually not associated with tissue proliferations. Further comparative studies on vein clearing and vein banding are needed for the elucidation of the nature of these symptoms.

Symptoms in the Phloem. The breakdown of the phloem as a conducting tissue may follow more than one distinct pattern. Tentatively these patterns are grouped as follows: *a*) degeneration begins with a necrosis of the sieve tubes themselves and is or is not followed by pronounced growth disturbances (*e.g.*, leaf-roll disease of potato, buckskin disease of peach and cherry, quick decline of citrus); *b*) degeneration begins with abnormal growth that is usually followed by more or less extended necrosis of the tissue resulting from this growth (*e.g.*, curly-top disease of beet, bunchy-top disease of banana); *c*) degeneration consists of a rather generalized necrosis affecting various phloem cells and often involving cells outside the phloem (*e.g.*, black-root disease of snap beans; chlorotic-streak disease of sugar cane).

The potato leaf-roll is the best known example of a disease whose most characteristic internal symptom is sieve-tube necrosis. The extensive literature dealing with this symptom has been fully treated by the writer previously (33). Workers generally agree that sieve tubes and companion cells collapse and that a yellow gum-like substance, which stains red with phloroglucinol and hydrochloric acid, appears in the walls and in the remains of the lumina of the collapsed cells. The most recent contribution on phloem necrosis in leaf roll by Sheffield (116) largely confirms the previous findings. According to Sheffield, the phloem necrosis in potatoes affected by leaf roll is distinct from phloem abnormalities induced by other pathogens or by physiological causes. It is restricted almost entirely to the primary phloem, and in plants grown from infected tubers (that is, in plants with secondary leaf roll) the amount of necrosis varies with severity of the disease but can always be found before leaf rolling is apparent. In newly infected plants (that is, plants with primary leaf roll) necrosis is mild and occurs near the bases of the lowermost rolled leaves and sometimes in the petioles. Regarding the details of necrosis, Sheffield does not specify that sieve tubes and companion cells become affected. She says that some or all of the cells of a group may be affected and speaks of a crushing of entire phloem strands. Such strands may be expected to contain sieve tubes, companion cells and phloem parenchyma. Thus, judging by Sheffield's statements, all three cell types may be crushed, but according to most references (see review by Esau, 33), necrosis is initiated in the sieve tubes. Minor growth disturbances appear to be associated with phloem necrosis in potato leaf roll. The parenchyma cells, which bring about the crushing of the necrotic cells, undergo hypertrophy, and the pattern of tissue formation by the cambium may be less orderly than in healthy plants (116).

The necrosis in the net-like phloem system of the tuber, that is, the "net-necrosis" of the potato-virus literature, has been definitely associated with leaf roll as a symptom of primary infection (43, 102). In other words, net necrosis appears in tubers formed by plants that have been infected during their growth. If, however, such tubers are planted and the plants that develop from them have leaf roll, the newly-formed tubers are free of net necrosis, even though the other parts of the plant show pronounced phloem necrosis. Although net necrosis is a good diagnostic symptom of primary leaf roll, Sanford and Grimble (102) think that some agents other than the leaf-roll virus may induce tuber-phloem necrosis. In one instance the authors traced net necrosis to a feeding injury by *Paratrioza cockerelli* Sulc. The fact that psyllid nymphs feeding on healthy plants induce symptoms resembling those of the virus diseases has been noted many years ago, and a toxin was suggested as the immediate cause of these symptoms (99). The occurrence of phloem necrosis in plants affected with psyllid yellows was also reported (39).

A precise characterization of phloem degeneration involving necrosis of the sieve tubes and companion cells has been supplied by Schneider (104) with reference to the buckskin disease of peach and cherry. The pathologic study is preceded by a detailed investigation of the normal phloem of the host plants (103). The necrosis of the sieve tubes and companion cells in buckskin-diseased trees resembles normal obliteration, but it affects elements which in healthy trees would still be functioning, and the necrotic cells contain wound gum. Identification of the latter is based on detailed microchemical tests, and "wound gum" is defined, in agreement with Hewitt (49), as a "water-insoluble substance which turns red when treated with phloroglucinol and hydrochloric acid but gives a negative reaction with the Maule test for lignin". The wound gum is present in the lumina and walls of the collapsed sieve tubes but sometimes also in the middle lamella of the adjacent parenchyma cells. The necrosis occurs in steps. The sieve tubes first lose their special wall thickening ("nacré", 34), then they develop callus masses on the sieve plates and finally they collapse and show presence of wound gum. At the beginning of the disease the oldest sieve tubes are affected, later also the younger ones. The new growth formed after these initial changes usually shows abnormalities. The sieve tubes and fibers fail to undergo turgor expansion, and the former do not develop the special thick walls and early become necrotic. The cambium may show a disturbance of the normal orderly arrangement of cells. Sometimes the parenchyma of the phloem and cortex shows hypertrophy and collapse of cells. In newly initiated vascular bundles primary rays may fail to develop, and the fiber initials may collapse. Depending on the strain of virus or on the degree of susceptibility of the host, cavities filled with gum develop in the phloem and cortex and sometimes also in the non-lignified new xylem.

Some hyperplasia of phloem also is associated with the buckskin disease. It occurs in the swollen veins of the affected leaves (98, 104). According to Schneider (104), such veins show necrotic sieve tubes and an abnormal production of secondary tissues, particularly phloem. This phloem contains mostly parenchyma, and its sieve tubes are crushed early.

Realizing that many agencies other than viruses may induce sieve-tube necrosis, Schneider (104) investigated the effects of ringing and of grafting on incompatible rootstocks on the phloem tissue of certain *Prunus* species. He found that these manipulations caused similar degenerative changes as the buckskin disease. He also observed that when a scion resistant to this virus (*Prunus avium*) was grafted on an apparently hypersensitive rootstock (*Prunus Mahaleb*), the presence of the virus in the tree caused a severe phloem necrosis in the stock near the graft union, and this necrosis led secondarily to an injury of the phloem of the scion. On the basis of his comparative studies, Schneider (104) suggested that the immediate agency inducing some of the symptoms in infected plants may be not the virus itself but some products of metabolism.

The quick decline of orange trees, recently identified as a virus disease (42, 131), is characterized by similar histologic symptoms as the buckskin disease (105). Quick decline induces sieve-tube necrosis, and the development of this abnormality is affected by the scion-stock relationship.

The phloem-necrosis disease of tea (16, 44, 45) may also belong to the group of diseases inducing sieve-tube necrosis. Details on the histologic symptoms, however, are lacking. Some phloem cells are said to be collapsed and yellow in color, and vein swelling occurs on leaves.

The virus-yellows disease of sugar beet, which was reported from Europe, shows sieve-tube degeneration described as gummosis by the two workers who studied the histology of the diseased plants (77, 100). The gum occurs mostly in the oldest sieve tubes near the outer limit of the functioning phloem; less commonly it may be found in the younger phloem region. A brown discoloration of the sieve-tube walls is sometimes associated with gummosis and may spread to adjacent cells. Growth disturbances seldom occur in this disease. Klinkenberg (77) reports that the gummosis of the sieve tubes is visible after the carbohydrate translocation is disturbed, that is, after the leaves show excessive starch accumulations. She concludes, therefore, that gummosis is not the cause of the impeded carbohydrate transport, but is, together with the latter symptom, a result of a diseased condition of the phloem.

The studies on the buckskin and quick decline diseases by

Schneider (104, 105) and on beet virus-yellows by Klinkenberg (77) suggest that premature death of the sieve tubes in some virus-diseased plants is not necessarily a direct result of the presence of the virus but may be a secondary symptom caused by disturbances in the metabolism of the plant. The statement of Bawden (6) that in the potato leaf-roll much starch and rolling of leaves are often found in the first year of infection when there is little or no necrosis in the phloem, points in the same direction. Wynd's (138, p. 429) suggestion, however, that the accumulation of starch before the appearance of any other symptom in tobacco leaves inoculated with tobacco mosaic (58) disproves a causal relation between phloem necrosis and carbohydrate accumulation, is misleading because tobacco mosaic induces no phloem necrosis. But apparently Wynd (138, p. 400) has the erroneous impression that phloem necrosis is "a phase of virus diseases for plants in general".

Among the virus diseases listed in the second group, that is, in the group characterized by growth abnormalities in the initial stages of phloem degeneration, curly top has been investigated in greatest detail. The studies on the histopathological effects of this disease on the sugar beet have been reviewed (33). The subsequent studies on tobacco and tomato affected with curly top (36) confirmed the previous findings regarding the details of phloem degeneration characteristic of this disease. As in the beet, the first degenerative changes in the solanaceous hosts become evident in newly developing organs after one or more apparently normal sieve tubes mature in the vascular bundles. Some hypertrophy but mainly hyperplasia is initiated in the vicinity of the first sieve tubes, and the new tissue strikingly deviates from the normal phloem by the over-abundance of sieve-tube elements which are usually of abnormal shape. As was pointed out previously (33), Artschwager and Starrett (2) interpreted the hyperplastic cells as intermediate between the sieve-tube elements and parenchyma cells because, in their opinion, these cells had no sieve plates or callus. The hyperplastic cells in tobacco, tomato and sugar beet (the latter was reexamined in connection with the tobacco studies, 36) have all the characteristics of sieve-tube elements in their structure and ontogeny, except that companion cells are associated with them only occasionally. Callose, showing a typical reaction to aniline blue, occurs in the abnormal sieve tubes (36). The hyperplastic tissue, especially that of the primary phloem, often collapses and, in tobacco, may or may not be followed by secondary hypertrophy and hyperplasia in the nature of wound-healing reactions. If no such reactions occur, internal cavities are formed in the plant.

Hyperplasia with production of supernumerary sieve-tube elements takes place in the external and internal primary phloem, in the secondary phloem and even in the parenchyma outside but adjacent to the phloem (36). The secondary phloem usually shows less collapse and necrosis, and forms a more orderly cell pattern than the primary. The phloem mother cells of the secondary phloem undergo more numerous divisions and produce more and smaller cells than they do in healthy plants. Moreover, sieve tubes arise not only from the fusiform initials of the cambium, but also from the ray initials. In other words, rays are substituted by rows of sieve tubes and parenchyma cells. Fibers usually fail to develop in the diseased phloem. The occurrence of hyperplasia in the secondary phloem of tobacco indicates that the stimulus to abnormal growth continues to be present in the phloem tissue as primary growth is supplanted by the secondary. It is not unlikely that the cambium itself is influenced by the virus, and recently Lackey (83) has reported the presence of the curly-top virus in the immediate cambial derivatives on the xylem side of tobacco stems. (Lackey calls this tissue "cambium", but in the photograph, 83, fig. 2, *D*, differentiating vessels are evident in the tissue illustrating the kind used for the test.)

Tobacco plants commonly recover from curly top (8, 128). In plants that have more or less recovered from the disease phloem degeneration progresses more slowly, fewer cells become involved and necrosis is light, but the remarkable phenomenon of increased production of sieve tubes is characteristic of curly-top tobacco, even if the disease is most mild (36).

Another disease in which degenerative changes in the phloem are initiated after differentiation of the first sieve tubes, is the bunchy top of banana. According to Magee (87), some hypertrophy and much hyperplasia occur next to these sieve tubes. All cells of the phloem, except the fully differentiated sieve tubes, respond to the stimulus, and thus an abnormal tissue develops in place of the usual phloem. The sclerenchymatous phloem sheath is also replaced by abnormal, usually hypertrophied, cells if the particular bundle is affected in its early ontogeny. Author does not state whether the hyperplastic cells differentiate into any specific type of elements but mentions that some of them are crushed by the hypertrophying cells. The contents of the hyperplastic cells and the crushed areas stain deeply, and Magee interprets the material having this avidity for stains as "mucilage" and compares it with the contents of the mucilage, or latex, ducts that occur outside the bundles in *Musa* (117). Since Magee (87) did not test the "infiltrating mucilage" microchemically, he failed to prove that the necrotic lesions are impregnated with latex rather than with wound gum or a similar substance. (Magee, 87, and earlier Skutch, 117, also describe "schizogenous mucilage ducts" in the outer periphery of the phloem—structures that in some of the drawings and photographs furnished by the authors suggest crushed protophloem elements.) The hyperplasia in bunchy-top banana leaves does not remain limited to the phloem but affects also the adjacent parenchyma (87)—a phenomenon considered in the part of this review dealing with symptoms in the parenchyma.

Growth disturbances initiated after the maturation of the first sieve tubes have been observed by McLean (92) in elm roots affected by phloem necrosis, a graft-transmissible virus disease (21, 123). Hypertrophy and hyperplasia occur near the first sieve tubes in the root tips, but in older roots and stems the sieve tubes themselves are necrotic. Moreover, in the stems the phloem is hyperplastic and mostly parenchymatous in nature. Thus, indications exist that the phloem degeneration caused by the elm disease might be similar to that in plants affected with curly top, but developmental details are lacking and the picture of the degeneration is still incomplete.

Even less detail is available on the degeneration of phloem in the leaf-curl diseases observed in South Africa (89). These diseases, like the Javan "Kroepoek" mentioned in the previous review (33), induce hyperplastic changes in the phloem. (Author calls the region of hyperplasia "pericycle", but the identity of the so-called "pericycle" and the outer phloem in the aerial parts of tobacco and many other plants has been sufficiently emphasized by the present writer, 33, 37.) The hyperplasia in the phloem results in the formation of new vascular bundles containing both phloem and xylem. The supernumerary bundles have a very irregular longitudinal course and resemble those occurring in the curly-top diseased sugar beet (31). Some necrosis and crushing of cells takes place in the hyperplastic tissue in leaf-curl plants. External leaf-like enations on veins are associated with the hyperplasia in the phloem.

According to an old reference not previously reviewed (101), excessive cell multiplication occurs in the phloem of tomato affected with the big-bud disease. The hyperplasia is said to be restricted to the internal phloem, but the article includes no illustration of a healthy structure for comparison with the one from a diseased plant. Growth disturbances might be the primary phloem symptoms in the Fiji disease of cane and of peach yellows. (See 33.)

A comparison of the virus diseases inducing primary growth anomalies in the phloem with those that cause early sieve-tube necrosis gives the impression that in the former the virus might be more directly concerned with the phloem degeneration than in the latter. The dependence of the initial disturbance on the presence of one or more mature sieve tubes even suggests that through these elements the virus is brought to the sight of degeneration. (See also 33.)

Diseases that have been placed in the third group regarding the effect upon phloem apparently produce much less characteristic abnormalities in this tissue than those of the first two groups. Necrosis does not seem to be specifically a phloem symptom and may even not start in this tissue. In the black-root disease of snap beans (67) necrosis is said to affect various cells of the phloem and also the cambium and the outermost layer of xylem. In the acronecrosis of potato, necrosis starts in the phloem and spreads to other tissues, particularly the xylem. (See 33.) The necrosis that develops in the streaked areas of the leaves of sugar cane affected with chlorotic streak (1) begins in the mesophyll and, after this tissue is destroyed, spreads to the vascular bundles. When the chlorotic areas are poorly defined in chlorotic-streak disease, gum-like deposits occur in the mesophyll, phloem and xylem cells. The purple-top wilt of potato, which is caused by the virus of aster yellows, induces necrosis of the phloem and later also of adjacent parenchyma (56, 84, 94). More detailed studies on the diseases just reviewed would perhaps reveal that the phloem necrosis they induce is similar to the type of degenerations classified with one or the other of the first two groups of diseases. Still less is known about the nature of phloem degeneration in several other virus diseases. Discoloration of vascular bundles is mentioned in the description of the sore-shin disease of blue lupine (93). In Savoy, a virus disease of the beet, discoloration and phloem necrosis were reported in roots of affected plants (26). Phloem necrosis is said to occur in certain virus diseases of cereals in Russia (139, 140, 141).

Symptoms in the Xylem. The best known virus diseases inducing degenerative changes in the xylem are those that are caused by the Pierce's-disease virus on the grapevine and alfalfa and by the psorosis virus on citrus. In both diseases the histologic abnormalties are not restricted to the xylem, but the Pierce's-disease virus must be introduced into the xylem before it can multiply and cause infection (61), wheras the psorosis virus is readily transmitted through bark grafts (129). It is possible, therefore, that the xylem abnormalities induced by the Pierce's-disease virus are primary symptoms, whereas those in psorosis-diseased citrus might be secondary. Psorosis induces characteristic extra-xylary symptoms—death of cells in the cortex and in deeper extracambial stem regions, followed by irregular periderm formation and an eventual scaly exfoliation of the bark—and these symptoms appear before those in the xylem (132).

The symptoms induced by the Pierce's-disease virus in its economic hosts, the grapevine and alfalfa, were described before the virus nature of the disease was discovered (50, 52, 134) and before the identity of the virus causing the Pierce's disease of grapevine and the dwarf disease of alfalfa was recognized (51, 54, 60). According to Dowlen (30), the wood of affected grapevine stems is discolored, many cell cavities are filled with black brown deposit, and tyloses occur in vessels, sometimes very abundantly. Butler (17) reported the presence of a darkly staining deposit in the xylem and other tissues and identified gum as one of the components of the deposit. He found that vessels were occluded with this substance and with tyloses in both leaves and canes (one-year-old shoots). The symptoms outside the xylem in Pierce's disease are uneven maturation of cane bark resulting from a partial failure of cork development (which normally occurs in the new shoots at the end of their first season of growth), scarcity of storage starch in the canes and degeneration of chloroplasts in the mottled leaves (17, 30). The only internal symptom that is on record in the literature with regard to alfalfa dwarf is the deposition of gum in the vessels (133, 134). The formation of gum and the consequent discoloration of the wood occur in the roots, the main divisions of the crown, and in the bases of the green stems, but apparently do not extend far up into the latter (133).

The psorosis disease is caused by a virus that is represented by five varieties (40). All these induce fundamentally similar alterations in the wood (14, 41). Duct-like spaces containing gum, partial plugging of vessels with gum and depletion of starch in the wood are characteristic of the affected xylem (132). Fawcett and Bitancourt (41) distinguish between primary and secondary lesions in the wood. The former develop very soon after or almost simultaneously with the lesions in the bark, occur near the periphery of the woody cylinder and consist of complete or partial concentric rings of duct-like spaces filled with gum. These ducts arise at the beginning of a season's growth and are followed by formation of apparently normal wood or of wood containing variable amounts of abnormal tissue composed of thin-walled but lignified, more or less isodiametric cells. The gummous degeneration generally involves cell complexes between the rays, and apparently occurs when these cells are still undifferentiated (41, 132). The gum ducts extend vertically for considerable distances, and the cells bordering them are frequently filled with gum. The latter may be present also in the vessels—usually only near the perforation plates—of the xylem layers that alternate with the layers of gum ducts. The secondary lesions occur in the older, more interior wood and commonly develop several years after the inception of the bark lesions and of the primary wood lesions (14, 41). The secondary lesions consist of irregular discolorations of the wood, with vessels partially or entirely filled with gum. Starch is absent in the discolored wood and the contiguous regions. The primary lesions retard water passage only partially; in the secondary lesions, however, movement of water is stopped completely (14).

Another disease that should be mentioned when symptoms in the xylem are considered is the phony disease of peach (62). The standard test for identification of this disease is the staining reaction of root xylem to methyl alcohol acidulated with hydrochloric acid: well distributed purplish spots appear in the xylem of diseased roots when they are treated with this reagent (62).

Gum pockets have been observed in the xylem of *Prunus* affected with the buckskin disease (104).

Judging from the literature just reviewed, the principal abnormality found in the xylem of virus-diseased plants is the formation of gum which eventually plugs the vessels. Whole cell complexes may break down and become decomposed into gum. Tyloses are sometimes associated with gum deposition in the vessels, but these structures probably arise in response to the plugging of the vessels with gum (97).

The formation of gum in the xylem is not a specific symptom of virus diseases. Other pathogens and certain physiological causes may induce similar gum deposition in this tissue. For example, gum accumulates in vessels of alfalfa plants invaded by the bacterium *Aplanobacter insidiosum* L. McC. (71, 72); of alfalfa roots suffering from winter injury (70) or from high water content of the soil (85). Introduction of salts and acids into the plant may produce a similar derangement (70, 85). In the grapevine gum formation occurs in apparently uninjured plants and is readily induced by mechanical wounding (97). Some workers consider that the gum in plants is derived from decomposed carbohydrates of the walls (*e.g.*, 18), others think that starch also furnishes much or perhaps most of the gum (*e.g.*, 5).

HISTOLOGY OF HOSTS AND THE PROBLEM OF CLASSIFICATION OF VIRUSES

As was detailed before (33), the use of symptomatology in the classification of viruses was tested most intensively with regard to the potato virus diseases. A new study in this direction by Bawden and Sheffield (7) again emphasizes that the clinical picture resulting from infection is as much a property of the host as of the virus. Related virus strains may produce widely different disease expressions on the same host, whereas unrelated viruses may induce very similar symptoms. Authors cite as examples that in potato of the variety Majestic one strain of virus-X causes top-necrosis and another a mottle; one strain of virus-Y causes top-necrosis (like one of the X strains) and another acropetal necrosis. Bawden and Sheffield (7) find, nevertheless, that a study of symptoms produced on a series of different hosts will tell whether the given viruses are identical or not, obviously because viruses causing different symptoms on the same hosts can not be identical. If the viruses are not identical, a study of their properties *in vitro* and of their immunological reactions must serve to determine whether such viruses are related or not. Among the potato virus diseases leaf roll appears to be rather unique in that its internal symptom, the phloem necrosis, is, according to Sheffield (116), very characteristic and useful in the diagnosis of the disease.

The discussion just presented does not distinguish between the external and internal symptoms, but, as was mentioned in the earlier review (33), the anatomic changes in virus-diseased plants are of a similar value in classification as the external symptoms, except that their determination is technically more complicated.

The internal symptoms thus continue to play a minor rôle in the delimitation of the smaller groupings of viruses. How useful they might prove to be in the establishment of the larger groups is difficult to predict because thorough information on the histologic effects of most virus diseases is still lacking. The available literature indicates that plants affected by the representatives of the two large groups of virus diseases, the yellows and the mosaics, may show significant differences in the internal symptoms. According to Bennett (9), these two groups of diseases have many distinguishing characteristics that appear to result from differences in the relation of the viruses to the tissues of the hosts. As was shown by the studies on the raspberry leaf curl and sugar-beet curly top (10), the viruses in the yellows group are more or less restricted to the phloem. In contrast, the mosaic viruses occur abundantly in both phloem and parenchyma. Curly top-diseased plants show phloem degeneration as a constant and primary symptom (33, 36), whereas the mosaics, as exemplified by those of the beet and the tobacco, induce no specific phloem abnormalities (inclusion bodies like those in the parenchyma may be present in this tissue) and their primary effects appear in the mesophyll of the leaves (36, 38). The value of such histologic differences in the classification of yellows and mosaics will depend, of course, on the degree of consistency of these distinctions in the various diseases of both groups. In addition to curly top, some other diseases classified as yellows by Bennett (10), namely, potato leaf roll, raspberry leaf curl and peach yellows, have been found to induce alterations in the phloem (see 33), whereas a degeneration of this tissue is rarely reported in connection with the mosaics. Of the newer papers that mention phloem necrosis in mosaic-diseased plants, one (139) deals with diseases whose infectiousness was not proven at the time of writing; the other two (140, 141) consider a rosette type of cereal disease transmitted neither by juice inoculations nor by aphides but by a leafhopper, *Deltocephalos striatus* L. On the basis of its method of transmission this type of disease does not seem to fit into the classification of mosaics given by Bennett (9).

Histologic papers dealing with viruses often lack the ontogenetic viewpoint, and a proper distinction between primary and secondary symptoms can hardly be attained without developmental studies. Such a distinction is very necessary because many virus diseases induce alterations in more than one kind of tissue, and consequently the tissue relation of a given virus may be obscured. Though in some diseases the viruses appear to be active in all tissues, in others the symptoms in one tissue may be a consequence of a disturbance in another primarily affected tissue. Phloem degeneration in curly top of beet and in bunchy top of banana is followed, secondarily, by growth anomalies in adjacent parenchyma. The primary visible degenerative changes in buckskin diseased peach and cherry occur in the phloem, but there may be gummosis of the xylem. In Pierce's disease the xylem appears to be affected primarily; in psorosis of citrus, however, the symptoms in the bark develop before the xylem alterations.

In summing up, the histological studies on virus-diseased plants do not provide a simple basis for classifying viruses and their relations to tissues, but, like the external symptoms and the cytologic abnormalities, the histologic changes have a place in any classification scheme, especially with reference to viruses that are otherwise difficult to distinguish.

ANATOMICAL ASPECTS OF THE PLANT-TISSUE RELATIONS OF CERTAIN VIRUSES

The plant tissue relations of viruses have been well reviewed in this journal (10), though chiefly from the physiological standpoint. Briefly, Bennett classifies the relations as follows: (*a*) virus is more or less restricted to parenchyma; (*b*) virus is more or less restricted to the phloem; and (*c*) virus occurs extensively distributed in both phloem and parenchyma.[3] No possible relation of a virus to the xylem tissue is indicated in Bennett's classification. The causal agent of the phony disease of peach, which so far has been associated with the xylem (62, 63), is placed by Bennett among those viruses that are more or less restricted to the parenchyma, in this instance to that of the xylem. The possibility that the relation of a virus to the xylem may be somewhat more specific than its occurrence and movement in the xylem parenchyma is suggested by the observations on the Pierce's disease virus (61). The causal agent of the Pierce's disease of the grape and of the dwarf disease of alfalfa is transmitted by vectors that seek out the xylem when feeding and it can multiply and cause the disease only when the xylem tissue is reached by the vector as it feeds. Experiments on alfalfa stems, moreover, showed, in some instances, a relatively rapid upward movement of the virus that suggested, in conjunction with the other evidence on the relation of the virus to the xylem, a transport with the water stream. Histological studies on affected hosts (17, 30, 133; see also part on symptoms in the xylem) indicate that the primary alterations occur in the xylem. Some of the external symptoms, namely, the sudden wilting of vigorously growing young vines and the scalding and drying of grape leaves (53), the wilting of stem tips and the dark coloration of the foliage in alfalfa (133) appear to be consequences of a derangement in the water-conducting tissue. If the transmission of the Pierce's disease virus and its effects upon the plant are compared with those of the curly-top virus (10, 31, 33), it seems that the association between the former and the xylem is as definite as the association between the curly-top virus and the phloem. The Pierce's disease virus probably lives and multiplies in the living cells of the xylem. This feature alone would make this virus no more parenchyma-limited than the curly-top virus: no evidence is available that a phloem-limited virus does not live and multiply in phloem parenchyma, even though it appears to move in the sieve tubes during its systemic invasion of the plant.

The possible relation of the virus to the sieve-tube protoplast is obscure. Bennett (10) suggests that viruses are released from the sieve tube cytoplasm and "occur more or less free in the lumen of the sieve tube" (presumably together with the vacuolar contents). This suggestion does not conflict with the concept of Crafts (28) that the cytoplasm of a sieve-tube element becomes permeable at maturity and that the interfacial boundary between the cytoplasm and the vacuole breaks down. The release of the virus from a permeable protoplast into the vacuole and its "rapid movement through the lumen of the sieve tube" (10) could be readily visualized. Sieve-tube contents, however, move not only through the lumina of the sieve-tube elements, but also from element to element across the sieve plate. Since the latter is supposed to be lined with cytoplasm and traversed by cytoplasmic strands (28), materials moving across the plate would be expected to pass through this cytoplasm. If virus movement in the sieve tubes is considered with reference to Crafts' (28) concept of sieve-tube structure and function, emphasis should be placed not upon the possible release of the virus from the cytoplasm into the vacuole, but upon the peculiar properties of the sieve-tube protoplasts which permit a mass flow of materials through it. If, however, the view is held that the vacuolar contents of two superimposed sieve-tube elements are continuous through the sieve-plate pores (34), then the virus could be pictured as having been released from the cytoplasm into the vacuole and moving within this medium from element to element.

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[3] In this classification "parenchyma" evidently refers to parenchyma cells outside the phloem, as those in the mesophyll, cortex, xylem, and elsewhere. The phloem, as a complex tissue, also contains parenchyma (phloem parenchyma and ray parenchyma) together with the sieve tubes and companion cells.

The mechanism of virus movement in the phloem is less difficult to explain than that in the xylem: in the phloem the virus need not leave cells containing cytoplasm, because even the sieve-tube contents exhibit some living properties. If, however, a virus should move naturally with the water in the tracheary element, one must assume a release of the virus from a living parenchyma cell into a dead element and a reentry into a living cell at some other point of its path; or, if the virus occurs in the treacheary elements only when it is placed there by the vector (according to Houston *et al.*, 61, the insect punctures the walls of these elements in feeding), its rapid appearance in parts removed from the place of inoculation would suggest an ability to enter uninjured parenchyma cells lining the water-conducting elements. Some workers who studied mosaic viruses deny their ability to pass in and out of tracheae whose walls are unbroken (see reviews by Esau, 33, and Bennett, 10), but perhaps some other viruses are capable of altering the normal properties of the protoplasts in such a manner as to make possible their own exit and reentry through the walls of tracheae; or the differential behavior of viruses with reference to a passage from one type of cell to another might be determined by the size of virus particles.

The precise agreement between the physiologic and anatomic studies on the curly-top disease, demonstrating the association of the causal agent with the phloem, has been sufficiently emphasized in the previous review (33). The new studies on the structural effect of curly top upon tobacco and tomato (36) confirm the findings on the beet and strengthen the concept that the virus moves in mature sieve tubes and causes primary degenerative changes in the neighborhood of these elements.

According to Bennett (10), viruses classified as those occurring in both the phloem and the parenchyma—mostly mosaic viruses—first invade plants through the phloem and later spread into other tissues where they continue to increase and often reach relatively high concentrations. As was mentioned in the foregoing part of this review, the primary histologic effects of mosaics, as exemplified by Johnson's tobacco mosaic strain number 1 and by the beet mosaic (36, 38), reside in the photosynthetic tissue, and the phloem may be free of any evidence of disease or may contain inclusion bodies of the same type as in the parenchyma cells. Thus it appears that though the mosaic viruses move in the phloem, they do not affect it as conspicuously as some of the yellows viruses.

Since the publication of Bennett's (10) review on tissue relations of plant viruses, a new method of transmitting viruses has further elucidated the problems of these relations. This method consists of a transfer of viruses from one plant to another through a stem of a dodder parasitizing on both plants simultaneously (11, 13, 68, 69, 79, 80). The anatomical aspects of the connection between the dodder plant and its host have served as a basis to explain the differences in the manner in which different viruses move through the bridge of dodder tissue. References on dodder-host connection agree about the existence of a thorough union between the xylem systems of the host and parasite, but they disagree on the relation between the phloem tissues of the two. Peirce (95) and Thoday (124) describe a direct connection between the sieve tubes of the host and parasite. Thomson (125), on the other hand, denies the existence of any phloem connection between the two organisms. Kindermann (76) and Schumacher (106) observed in certain dodder species that elongated hyphae-like haustorial cells—the outgrowths from the haustorium (adventitious root) anchored in the host tissue—come in contact with the sieve tubes of the invaded plant. The species of dodder used by Bennett (13) brings similar haustorial cells in contact with the sieve tubes of tobacco and beet, and these cells are continuous, within the haustorium proper, with the sieve tubes of the parasite. Regarding the relation of the dodder cells to the parenchyma of the host, Peirce (96) has reported that the haustorial cell penetrates the parenchyma cells of the invaded plant apparently without disturbing their protoplasts, and, according to Schumacher and Halbsguth (107) and Bennett (13), the walls of such haustorial cells show, upon treatment with plasmodesmata stains, numberless fine lines that project from the protoplast of the parasite into the walls and seemingly come in contact with the protoplast of the host. Thus, judging from the literature on dodder-host connection just reviewed, one could expect: (*a*) complete xylem continuity between the host and parasite; (*b*) a connection between the sieve tubes of the two organisms, perhaps not a direct one but through specialized cells capable of withdrawing materials from the host phloem; (*c*) a possible contact of parenchyma protoplasts of host and dodder through plasmodesmata in the walls of the latter. (Bennett 13, considers also the possibility that the tips of the haustorial cells may be without walls so that the protoplasts of dodder and host come in direct contact. Presumably he relies on the report of Thoday that the walls of the hyphae-like cells approaching the phloem of the host become mucilaginous.)

Bennett's (13) summary on all the work done so far on transmission of viruses through dodder suggests that viruses are acquired by the dodder mainly by movement from the host into the parasite through the phloem with food materials; that infection of a new plant by a yellows type of virus occurs by movement of the virus from the phloem of the dodder through the haustorium into the phloem of the host against the prevailing direction of food movement; that the transfer of a mosaic virus into a new plant is effected by passage from the parenchyma of the haustorium into that of the host.

The concept of virus movement through plasmodesmata in the parenchymatous tissues continues to be conjectural. Sheffield's suggestion that tobacco mosaic did not enter guard cells—as evidenced by the supposed absence of inclusions in these cells—because the guard cells and the adjacent cells were not connected by plasmodesmata (see 33), found no support in subsequent observations. Guard-cell walls were shown to have plasmodesmata, at least when they were somewhat young (35), and mosaic inclusions were found in these cells (33, 35, 74). Inclusions associated with certain other virus diseases also were encountered in the guard cells (73, 114).

New evidence has been obtained by Martin and McKinney (88) that tobacco-mosaic virus occurs in the cytoplasm rather than the vacuole. The authors separated the vacuolar sap from the cytoplasmic extract by suitable means and compared the infectiousness of the two with a preparation of highly purified virus protein after a dilution to a uniform protein content of all three preparations. The diluted samples of the vacuolar sap, the cytoplasmic extract and the control induced 1, 240 and 222 necrotic lesions, respectively, on 32 bean leaves each. These results indicated a more definite localization of the virus in the cytoplasm than the earlier work of similar nature of Livingston and Duggar (see 33). Authors conclude that this evidence makes it simple to explain the movement of virus from cell to cell: the latter occurs through plasmodesmata which are visible through a microscope and hence are sufficiently large to allow passage of the submicroscopic virus particles.

Regarding the actual size of plasmodesmata, Livingston and Bailey (86), who were studying these structures in unaltered walls of living cells, reported that their diameters were not much more than 0.2 micron in the various tissues of plants. Authors' statement that "the threads penetrating the walls from adjacent cells are intimately associated with one another if not actually continuous" implies, however, that plasmodesmata are not necessarily continuous between two cells.

The evidence that virus is pretty clearly limited to the cytoplasm bears on the matter of transfer of viruses through graft unions.

The passage of virus from scion to stock occurs only after the actual union (which probably brings about similar cytoplasmic unity as exists in an unbroken tissue) between the tissues of the two plants has occurred (12). Moreover, the time of contact between scion and stock that must elapse before a virus can move across the graft union is much affected by the virus nature. A mosaic type of virus requires a shorter contact period than a representative of the yellows group (12, 78) because a mosaic virus can pass when parenchyma cells of scion and stock are united, whereas a yellows virus requires the establishment of phloem continuity (12). The latter, of course, develops later than the junction of parenchymatous tissues (27).

Some viruses have been transmitted by placement of pieces of leaf tissue from diseased plants under bark flaps of healthy plants (22, 120, 130). Wallace (130) has shown that such transmission of the psorosis virus of citrus was successful because an organic union was formed between the leaf tissue and the tissues of the stem. Callus was produced on the leaf piece and united with the callus that developed on the bark and wood side of the stem. Wallace's observation suggests that a protoplasmic union is necessary before psorosis virus can move from the leaf piece into the new host plant.

Considering the distribution of viruses in different parts of the plant, some investigators have attempted to determine whether viruses occur in meristems. Bennett (10) thoroughly reviewed the literature that appeared on this subject before 1940 and concluded that evidence of cytological nature indicating presence of virus in cells of the meristems seems to be lacking and that the continued normal functioning of meristems of most virus-affected plants indicates that if viruses are present in the meristems they rarely injure them. To obtain cytological evidence of virus occurrence in meristems, workers have centered their attention on the distribution of inclusion bodies in the youngest plant parts, and these structures seem to begin development in partly differentiated regions subjacent to the apical meristems (114, 115). Lately some efforts have been made to determine the possible presence of virus in the meristems directly, that is, by testing the infectiousness of the juice extracted from the meristems mechanically or by means of the vector. Sheffield (115) thus obtained a positive test on the occurrence of virus in the apical meristems of roots and shoots in plants affected with tobacco and aucuba mosaics, and Lackey (83) reported relatively high concentration of curly-top virus in the apices of roots of the beet and tobacco and in the cambial region of tobacco.

Although the meristems of virus-diseased plants commonly show no microscopic evidence of direct injury to them, the general validity of the statement about the usually "continued normal functioning" of such meristems (10) may be questioned. Many virus-diseased plants are severely stunted and their organs are often much malformed. There is no simple way of determining at what level in the growing region of a plant the rate and course of development of the tissues is controlled, but the recent studies of Ball (3) indicate that such control may reside in the apical meristem. By dissecting out pieces of stem tips of *Tropaeolum* and *Lupinus* and growing them in sterile cultures, Ball found that the shoot apex possesses the capacity for development of the entire plant in a much higher degree than the subjacent tissues. The pieces including the apical meristem were able to grow into entire plants in tissue cultures, whereas the partly differentiated subjacent stem pieces produced only parenchymatous masses with few or no tracheary elements. Author concludes (3) that the "shoot apex is not merely a plastic region that obeys the impluses sent to it from the mature, subjacent regions of the plant, but actually controls or 'determines' the growth and behavior of the subjacent regions." In view of Ball's observations, the dwarfing and the deformation of virus-diseased plants may be indications of a serious injury to the meristems. Lack of distortion of plant parts, on the other hand, may signify that virus does not injure the apical meristem or is perhaps absent from it (126).

The question concerning the occurrence and non-occurrence of viruses in meristems is intimately related to the problem of seed transmission of viruses. The various aspects of this problem have been reviewed by Bennett (10) and Esau (33). The final explanation of the usual absence of transmission of viruses through seeds is yet to come. In the meantime, authors continue to supply evidence that viruses may be unable to invade the generative cells or are destroyed before the seed matures. Gratia and Manil (48) confirmed their previous report (see 33) that in the differentiating floral parts the tobacco mosaic virus becomes less concentrated and finally disappears, and that this happens long before the sexual cells are formed. According to Kausche (75), the tobacco mosaic virus is present in unripe seeds and sometimes in the dried seeds but is inactivated by a substance that is elaborated during the progressive ripening and germination of the seeds. Sheffield (114) found that, though the inclusions in severe etch (a disease not transmitted through the seed) are more widespread in the tissues and cells of affected plants than those induced by any other virus so far recorded, they are not present in the tapetum, the pollen mother cells, the pollen grains, stigma, young ovules, embryo sac, endosperm and young embryo. The absence of inclusions from the parts just named is the more striking, as their occurrence has been recorded (114) in the filaments and anthers of the stamens, in the ovary wall, the style and the seed coat.

Despite the common view that meristems are little or not at all injured by viruses (10), the relatively high susceptibility of young plant organs to virus infection, as compared with the old ones, is well known (*e.g.*, 59). Many workers have pointed out the difficulty with which symptoms develop in fully formed plant parts, and some strongly emphasized that, at least in the mosaic types of viruses, the principal effect of the virus is suppression of development, rather than a destruction of existing structures. (See part on symptoms in the parenchyma in the present review.) Good evidence has been now obtained, however, that old leaves also can develop symptoms—not only local, but systemic symptoms as well—if virus movement into such leaves is assured. It has been well established for many viruses that their prevailing movement occurs with the food toward the growing regions. But a virus can be inoculated into an old leaf and then it may induce symptoms other than the local lesions in such a leaf. According to Smith (118), a yellow strain of tomato streak produces local yellow spots on the mature inoculated leaves of White Burley tobacco within four or five days after inoculation. Valleau and Diachun (127), using a strain of white mosaic virus of tobacco, observed virus multiplication in and yellowing of inoculated leaves which were fully grown. They also obtained a slow movement of the virus into old uninoculated leaves where prominent symptoms developed in the invaded portions of the leaves. Hildebrand and Curtis (55) forced the virus of Yellow Red disease of peach into mature as well as growing leaves and obtained symptoms on both by darkening the leaves and thus directing the flow of food and virus into them.

McKinney and Clayton (90) made a detailed study of the relative susceptibility of leaves of different ages in tobacco infected with the yellow tobacco mosaic. They found that leaf tissues in very early stages and very late stages of development were more resistant than those in the intermediate stages. They regarded leaves that had completed about 35 to 40 per cent of their growth as the most susceptible and concluded that the type of reaction to the disease in a given leaf was determined by the level of resistance attained by the leaf at the time the virus reached it.

In studying the distribution of the white-mosaic virus in tobacco, Valleau and Diachun (126) have found clearly expressed localization of the virus in the yellow areas of the inoculated and the non-inoculated, but later invaded leaves. Similarly Storey (122) observed that the insects were able to pick up the virus of the streak disease of maize only from the chlorotic areas of the leaves. Selman (108) reported absence of systemic infection and a localization of the tobacco-mosaic virus in the developing tomato fruits when the virus was inoculated into individual leaves of fruiting plants. Such examples of localization of viruses in the process of the so-called "systemic" invasion of plants and the information on the more or less complete restriction of certain viruses to a single type of tissue (10) raise the question concerning the exact meaning of the terms "systemic infection" and "systemic symptoms". Since the absence of virus from one or another group of cells or type of tissue is so common, the restriction of the term "systemic" to those conditions in which the virus is shown to invade every cell of the organism will, indeed, severely limit its application, if not cancel it entirely. The continued use of the word "systemic", implying an establishment of the virus in the plant through multiplication and more or less thorough invasion of the tissues, in which the virus is characteristically present, seems desirable to the present reviewer.

CONCLUSION

The value of anatomic studies in increasing the understanding of the nature of virus activity in the plant has been well illustrated by the literature of the past decade. The recognition of the distinction between the phloem-limited viruses of the yellows group and of the mosaic viruses that occur in both the phloem and the parenchyma, though obtained largely through a physiologic approach to the problem, has been materially aided by the consideration of the anatomic aspects. The studies on the phloem alterations in virus-diseased plants have shown that the effects of viruses on the same tissue may differ strikingly: some viruses induce growth anomalies which seem to be more directly associated with the presence of virus than the necrosis of the sieve tubes induced by some other viruses. Anatomic studies on woody species have assisted in the recognition of some diseases as virus diseases. The knowledge of the effects of viruses on plant cells has been enhanced by the observations that not only do viruses inhibit tissue differentiation but also cause a destruction of fully formed cell constituents. Finally, the recent anatomic studies have brought abundant proof that the type of alterations induced in plants by viruses depends upon the reactions of the host as well as upon the activity of the virus, and that these reactions are usually fundamentally similar to those released by various agencies other than viruses.

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PALEOZOIC SEEDS II[1]

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INTRODUCTION

In an article published in The Botanical Review in 1938 (4) the present author described the preservation, classification, structure and affinities of the seeds of Paleozoic plants. The present paper incorporates some literature on seeds that was not included in the previous one, and an effort is made to review and appraise those investigations that have been reported since 1938. The treatment is not as thorough and complete as might be desired because many of the European publications since 1939 are not yet available, and the contents of some of the latest seed literature is known to the author only through summaries and reviews.

THE CUPULATE OVULE

The oldest known phanerogams bore their seeds in husklike cupules at the tips of small branches or frond ramifications. Although the cupules of different plants differ considerably in size, most of them were small, ranging from two to five millimeters in diameter and 10 to 20 millimeters in length. Some were larger. The cupule was constructed of a whorl of four to six slender pointed bracts that were usually united laterally for one half or more of their length, with the tip portions free. The ovule was situated deep within the cupule. Before pollination it was completely or almost completely enclosed, but as it matured, the bracts split apart from the tip downward, allowing the seed to fall out. Cupules from which the seeds had escaped sometimes resemble four- or six-rayed stars. On some of the cupule-bearing plants the pollen was borne in slender sporangia in terminal tufts that closely resemble the separated bracts of the seed cupule, and unless pollen or seeds can be demonstrated in connection with the fructification, it is often impossible to distinguish the two organs in the fossil condition. Because of the impossibility of distinguishing some cupules with split segments from tufts of slender pollen sacs, it can not be accepted as a matter of certainty that all cupules bore seeds. It is possible that some pollen sacs were borne within cupules, and in the yet undiscovered cryptogamic ancestors of seed plants, the spores might have been produced in a similar way.

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[1] Supplement to article in The Botanical Review 4: 205–234. 1938.

The cupulate fructification first appeared in the Upper Devonian, but no seeds have been found in cupules older than Lower Carboniferous. Whether the Devonian cupules bore seeds or spores is not known. No seeds of any kind have yet been found in the Devonian, and the supposition that seed plants were in existence during that period is based entirely upon indirect evidence. *Eospermatopteris,* originally described as a Devonian seed plant, was in all probability a spore bearer. The bodies thought to be seeds, but which were not found attached to the foliage, were shown several years ago to be spore cases. The leafy branches attributed to *Eospermatopteris* are indistinguishable from those of *Aneurophyton,* and since both occur in rocks of the same age there is no good reason for maintaining the name *Eospermatopteris* over which *Aneurophyton* has priority. *Callixylon* and *Palaeopitys,* both from the Devonian, are believed to be seed plants, but the evidence is limited to vegetative structures.

In 1935 the present author (3) described a pair of detached husk-like fructifications from the Upper Devonian of Pennsylvania associated with fertile and vegetative remains of *Archaeopteris.* Although the possibility of this being the seed husk of *Archaeopteris* was entertained at the time, more thorough examination of the associated spore cases of *Archaeopteris* revealed distinct micro- and megaspores (6, 7). The cupule obviously belongs to some unknown plant.[1] Other cupulate organs have been reported from the Devonian (2), but no seeds or pollen have been found within them.

Cupule-borne seeds appear characteristic of the lyginopterid group of pteridosperms of which *Lyginopteris oldhamia* is the most completely known member. It was because of the occurrence of the same sort of glands on the husk and vegetative parts that the identity of the parts was proved. It is possible that cupules were borne by pteridosperms of other groups, but it is impossible in the light of our very imperfect knowledge to specify those that bore them and those that did not.

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[1] In a paper received after this manuscript had been submitted for publication, Kräusel and Weyland (Paleont. 86, B: 36. 1941) compare this cupule with the fructification of *Rhacophyton incertum* found in West Virginia. The present author accepts this interpretation as entirely possible.

The nomenclature of cupulate and tufted fructifications is confused because many of them were only briefly described and crudely figured. The names most commonly applied to these organs are *Alcicornopteris* (28), *Calathiops* (21), *Calymmatotheca* (39), *Lagenospermum* (32), *Lagenostoma* (35), *Pterispermostrobus* (38), *Schuetzia* (20) and *Telangium* (8). Some of these names, *Telangium* for example, were originally given to pollen-bearing organs. Others were intended for ovulate cupules, and still others were for structures of indeterminate nature. Some which were originally thought to have borne pollen have since been found to contain seeds.

The most frequently encountered fructification believed to be seed-bearing is the type usually assigned to Stur's genus *Calymmatotheca* (39). Stur found no seeds associated with or attached to these cupules, and he made no reference to them as seed-bearing. In fact, he believed them to be indusia of ferns and classified them under the Cyatheae. Contrary to some later authors, he did not call *Calymmatotheca* a spore case. His figures show a leafless branch system, the ultimate ramifications of which bear terminal cupules consisting of four to six slender pointed segments which are separate to the base. Before maturity the organ apparently was closed except for a small opening at the tip.

The first intimation that *Calymmatotheca* was seed-bearing seems to have come from Kidston (29) who identified material from the Carboniferous Limestone as *Calymmatotheca Stangeri*. It was evident to Kidston that the free segments were portions of the dehisced wall of a closed structure and not elongated spore sacs of the *Telangium* type which look much like *Calymmatotheca* when preserved as compressions. Kidston also saw small, oval, seed-like bodies among the compressed cupules. According to Benson (8), Kidston's interpretation of *Calymmatotheca* has been upheld by reexamination of Stur's type material.

The cupule that contains *Lagenostoma Lomaxi*, the seed of *Lyginopteris oldhamia*, would, in the compressed condition, be referable to *Calymmatotheca*, and because of priority this is the generic name now applied to the plant of which *Lagenostoma Lomaxi* is the seed and *Lyginopteris oldhamia* the stem. The name *Lagenostoma* was first given to petrified material, so in 1914 Nathorst (32) proposed *Langenospermum* for cupulate seeds that resemble *Lagen-*

ostoma but are not petrified. Although *Calymmatotheca* has priority over *Lagenospermum,* the former was not originally applied to fructifications known to contain seeds, and some authors prefer the latter name when seeds are present. The present author chose *Lagenospermum* for some seed-bearing cupules from the Mississippian of Pennsylvania and Virginia in 1939 (5). These cupules are on slightly unequal stalks resulting from a bifurcation a short distance from the tip. The cupules are about three mm. wide and nine to ten mm. long, and consist of at least five slender bracts joined basally for about two-thirds of their length. The single seed is about two mm. in diameter and five mm. long. The cupule stalks, as in other cupulate forms, are entirely devoid of foliage, and it would be extremely difficult to homologize the seed parts or their surrounding bracts with leaves, as some investigators have tried to do. These parts show no relation whatsoever to leaves. This fructification occurs in the lower Mississippian Pocono and Price formations, which lie directly upon Upper Devonian strata, although they are separated by an unconformity. These cupules are among the oldest known seed-bearing organs.

In 1863 Geinitz (20) established the genus *Schuetzia* for a stout straight axis bearing alternately arranged tufted or cupulate appendages. His figure shows five on one side of the axis and eight on the other. Geinitz did not determine whether these side organs bore spores or seeds, and no views were expressed concerning their morphology until Kidston (29), after studying British material which he named *Schuetzia Bennieana,* concluded that the organs were microsporangiate. The same material was reexamined by Benson ten years later, and her observations and conclusions are given in a series of short articles (10, 11, 13). Unfortunately, her account is vague and requires careful reading, and one does not feel sure that the facts she presents justify her conclusions.

In Kidston's material of *Schuetzia Bennieana,* Miss Benson saw spores which she thought were immature embryo sacs. She therefore concluded that the fructification was ovulate, although she found nothing resembling completely developed seeds. In one of her notes of 1934 (10) she figured a tetrad surrounded by disorganized carbonaceous material of the cupule and sporangium. This tetrad measured 20 microns in diameter, and apparently was found in one of the specimens Kidston had described earlier, but since

Kidston had given the diameter of the spores of *S. Bennieana* as 50–70 microns, Miss Benson assumed that the smaller spores found by her were undeveloped ones and that the larger ones that Kidston saw were mature embryo sacs. In a paper published the following year (13) Miss Benson gives a few additional details and compares her supposed immature embryo sac spore with the pollen grain attributed to *Heterangium Grievii* and which she also believed to belong to *S. Bennieana.* She notes the similarity in size of the two and apparently considers this ample evidence of identity. The size resemblance, she says, supports Thomson's contention that seed plants are homosporous instead of heterosporous, as commonly believed. This matter will be considered further on a later page.

In presenting her case for the ovulate nature of *Schuetzia Bennieana,* Miss Benson relies too much on assumptions, one being that the spores found by her and by Kidston belong to the same kind of plant. Kidston explicitly states that the spores he attributed to *S. Bennieana* were not found in the same specimens he described and figured, but came from other material. While stating that he felt no doubt about their identity, he does admit an element of uncertainty, owing to the fragmentary nature of the fructifications containing the spores. As a result of the differences in spore size, as reported by the two investigators, the logical conclusion is that spores belonging to different kinds of plants were described and that the spores Kidston believed to belong to *S. Bennieana* represent some other plant.

Another reason for questioning Miss Benson's interpretation of *Schuetzia Bennieana* is that the only apparent reason for believing that the fructification bore seeds is that only one spore tetrad was found. This tetrad was observed in sectioned carbonized material in which the structure of the tissue surrounding the tetrad had broken down. She mentions the tetrahedral arrangement and the thick cuticle of the spores. If we assume that Miss Benson was correct in her assumption that only one spore tetrad was present, proof is still lacking that the spore developed into an embryo sac. It is not impossible that during the early evolution of the seed a single spore tetrad survived at the expense of the others in the sporangium, and from this tetrad one spore matured which was discharged and germinated outside. In some of the free-sporing

Paleozoic lepidodendrids a single large spore developed within the sporangium.

One may also question the affinity of the pollen which Miss Benson compared with the spores she believed to represent the immature embryo sacs. The pollen used for comparison was not from the same source as the other spores, but came from petrified material of a fructification she had named *Heterotheca* (9) and which she believed to be the microsporangiate organ of *Heterangium Grievii.*

Although Miss Benson's conclusions concerning the ovulate nature of *Schuetzia Bennieana* are interesting and suggestive, they seem to lack proof and to depend too much upon assumed identity of parts which probably belong to different plants. Further investigations are necessary before one can be certain about the nature of this particular fructification.

In 1865 Goeppert (21) proposed the name *Calathiops* for tufted fructifications and mentions the possibility that they might have borne seeds. The name was generally overlooked until Gothan (22) utilized it in 1927. In 1935 Benson (12, 14) gave a revised diagnosis of *Calathiops* and transferred *Schuetzia Bennieana* and the fructifications of *Sphenopteridium affine, S. bifidum* and *Diplopteridium Teilianum* (the latter three having been previously assigned to *Telangium*) to it. Her revised diagnosis is based partly upon *Calathiops Bernhardti,* from the Namurian of Westphalia, in which several cupules were crowded together on short pedicels. *Calathiops* and *Calymmatotheca* are both ovulate, according to Benson, but the latter is distinguished by its elongated pedicels. Its individual cupules appear to stand free and apart from each other. *Calathiops* and *Calymmatotheca* may therefore, Miss Benson claims, be merely developmental stages of the same fructification.

In 1931 Walton (44) described as *Calathiops Renieri* some large cupules from the Lower Carboniferous. They are tulip-shaped organs about 4.5 cm. long and 2.7 cm. wide. No seeds were found within them, and the plant on which they were born is unknown.

Probably the largest cupule ever described is *Megatheca Thomasii* which Andrews made known in 1940 (1). The only specimen found measures 6.2 cm. in length and 2.3 cm. in width near the base. It consists of six leathery pointed lobes joined at the base but free for the upper three quarters of their length. The basal portion of each lobe is ornamented with a network of hypodermal sclerenchyma which becomes less pronounced upward and which disappears entirely about half way to the apex. No seeds were found, and the assumption that the cupule bore ovules is inferred from its resemblance to others that do.

A fructification similar to *Megatheca,* but in which ovules were found, is *Calathospermum scoticum,* briefly described by Walton (45). Like *Megatheca,* it consists of six partly joined lobes, but the whole structure is slightly shorter. Each lobe has four to six vascular strands, and the centrally placed stalk of the cupule has in cross section a crescentric vascular strand which suggests that the organ is part of a leaf complex. There are several stalked seeds within the cupule, some of which are attached to the base and others to the edges of the lobes. Each seed has a long nucellar beak, recalling *Trigonocarpus,* but the integument apex is prolonged into nine narrow projections forming a terminal tuft similar to *Gnetopsis.* Some of the cupules contain no seeds, but their enclosed stalks extend to the top. It seems likely that these stalks extruded the seeds at maturity. *Calathospermum* and *Megatheca* are similar organs, probably belonging to closely related plants.

The following is a synopsis of cupulate and tufted Paleozoic fructifications which will aid in understanding the various types:

I. Cupules or tufts small; single-seeded if seed-bearing.

1. *Calymmatotheca* (Stur, 1877). Cupules on elongated pedicels, borne singly; type specimen apparently seed-bearing but seeds not observed. Cupulate seeds of the *Lagenostoma* type are now considered referable to *Calymmatotheca.*

2. *Lagenospermum* (Nathorst, 1914). Name proposed for *Lagenostoma*-like compressions in which a seed is present. *Calymmatotheca* has priority if it has been correctly interpreted.

3. *Calathiops* (Goeppert, 1865). Differs from *Calymmatotheca,* according to Benson, by its clustered shortly-stalked ovules; regarded as possibly seed-bearing by Goeppert, and ovule-bearing by Benson.

4. *Schuetzia* (Geinitz, 1863). Cupules arranged in spicate inflorescences; pollen-bearing according to Kidston, ovulate according to Benson. Single detached cupules are indistinguishable from *Calymmatotheca.*

5. *Telangium* (Benson, 1904). Terminal tufts of elongated spore sacs; undeterminable in the compression state unless there is evidence of the presence of pollen.

6. *Alcicornopteris* (Kidston,1887). Synangial or campanulate organs on short bifurcated stalks; believed by Kidston to bear pollen, but pollen not described. Characterized by its straight or coiled aphleboid foliage. The detached organs are probably referable to *Telangium* if pollen is present.

7. *Pterispermostrobus* (Stopes, 1915). Cupules with unknown contents on forked pedicels; resembles *Calymmatotheca* and *Lagenospermum.*

8. *Xenotheca* (Arber and Goode, 1915). Tufted or campanulate organs with unknown contents; from the Devonian.

II. Cupules large; many seeded where known.

9. *Calathospermum* (Walton, 1940). Six or more seeds per cupule.

10. *Megatheca* (Andrews, 1940). Seeds unknown; probably congeneric with *Calathospermum.*

THE QUESTION OF HETEROSPORY

Most botanists regard heterospory as characteristic of all seed plants. The pollen is presumed to develop from a microspore and the embryo sac from a megaspore. The process of the evolution of the seed is alleged to have commenced with an ancient homosporous form which gave rise to heterosporous types. The seed then evolved from a megasporangium. In the teaching of morphology and plant evolution *Selaginella* has long been used to illustrate what is usually believed to have been the essential heterosporous stage in the transformation from the strictly cryptogamic condition to the seed habit. Even though it may be apparent to most botanists that *Selaginella* represents a trend apart from seed plant evolution, its appropriateness for this purpose has been taken for granted, and its use justified on grounds that it is the replica of the stage through which the ancestor of the seed passed during its process of becoming a seed. Since *Selaginella* is regarded as an advanced crytogam on the verge of becoming a seed plant, it is usually placed above the ferns, which are looked upon as more primitive because of the general homosporous condition, and below the cycads. The more basic fact that it is merely a specialized member of an otherwise primitive group is generally ignored. Where, some will ask, can one find a better example of the heterosporous condition essential in the evolution of the seed than *Selaginella* with its microspores and megaspores?

Although *Selaginella* can be looked upon as an example of a directional trend within the Lycopsida, the heterospory it so strikingly exhibits is an ancient feature. In the Carboniferous *Lepidocarpon* the seed habit is virtually achieved, but no one believes that *Lepidocarpon* was in line with or had anything to do with evolution among the higher seed plants, or that the ancestors of seed plants passed through a lepidocarp stage. Moreover, there is no evidence that the lepidocarp line gave rise to any higher types. *Lepidocarpon* merely illustrates the principle so apparent in the plant kingdom that similar characteristics often develop independently of each other.

Heterospory in *Selaginella* has probably exerted more than its rightful share of influence on our concept of the spore condition in seed plants. The homology between the spore and a cell that forms an embryo sac dates from the classical researches of Hofmeister, but the fact has been almost universally overlooked that in making the comparison Hofmeister did not compare the embryo sac with a megaspore or the pollen grain with a microspore, as he is commonly supposed to have done. Instead he merely said: "The embryo sac of the conifers may be regarded as a spore which remains enclosed within the sporangium". He makes no reference to any difference in size between the spore that develops into the embryo sac and that which forms the pollen grain.

Twenty years ago Thomson (41) assembled all of the available information on comparative sizes of the so-called microspores and megaspores in seed plants. He found that in both gymnosperms and angiosperms, where actual measurements are on record, the pollen spore is as large as or larger than the seed spore in about as many instances as where the seed spore is the larger. Moreover, in those instances where the seed spore is the larger, it does not exhibit the enormous difference in bulk shown in the heterosporous lycopods. Thomson also calls attention to the perfectly obvious but often overlooked fact that the embryo sac at the fertilization stage, where it displays its maximum bulk, is not comparable to the cryptogamic spore. At this stage it is a gametophyte comparable, as concerns its place in the life cycle, to the mature prothallium of the fern.

Thomson concludes (41–43) from his comparative studies of the sizes and other characteristics of pollen and seed spores that seed plants are homosporous and have evolved from homosporous ancestors. He finds no evidence that there ever was a heterosporous stage in seed plant evolution. Heterospory among living plants prevails only among some of the lycopods and specialized ferns. It is heterothally, not heterospory, that constitutes the distinctive feature of seed plants.

This rather revolutionary and extremely unorthodox view that Thomson has espoused toward the spore condition in seed plants is important because of its bearing upon questions pertaining to the origin and early evolution of the seed. Benson (13) says that the spore condition found by her in *Schuetzia Bennieana* supports Thomson's views about recent plants. Under the notion that heterospory is characteristic of the seed habit, it has always been assumed that the seed would have been preceded by a heterosporous stage. This assumption is well stated by a pertinent remark of Halle (24): "The Pteridosperms are generally held to have sprung from Filicinean stock. Perhaps the greatest difficulty in trying to trace a connection between the two groups is the absence of reliable records of any kind of heterosporous Paleozoic ferns that might have formed a starting point for the evolution of the seed habit". He also mentions the possibility of the seed having developed in a terminal position from the psilophytalean spore case, a possibility that finds support in the terminal position of the oldest known seeds. Halle's statement leaves two alternatives: one that heterosporous ferns existed but await discovery, the other that the seed was derived from a homosporous ancestor. As will be explained in the following paragraph, heterosporous fernlike plants did exist during the Paleozoic, but the question of the origin of seed plants from a heterosporous ancestor is still an open one.

A few years ago the present author (6) found spores of two distinct sizes of the Upper Devonian genus *Archaeopteris*. The microspores were numerous and small and the megaspores were large and few. The differences with respect to both number and size were such that they can not be merely variations of one type. It is a case of well developed heterospory. Having held at the time the prevailing view about the spore condition in seed plants, the author suggested that this particular specimen of *Archaeopteris* might provide a "missing link" in the evolution of the early seed plants (7), and might also supply the necessary heterosporous stage of the derivation of the seed from a cryptogamic sporangium.

Although it may be necessary to modify our notion of the rôle of *Archaeopteris* in seed plant evolution, the fact must be kept foremost that unisexual gametophytes did have to appear at some stage, and that this was far more significant than mere size differentiation among spores. This segregation of the sexes in the gametophytes resulted in heterothally. Different kinds of gametophytes can grow from spores that are superficially alike (isospores), but microspores and megaspores can not produce bisexual gametophytes. Therefore heterospory in a fossil fern supplies the assurance that heterothally also existed. In some ancient ferns heterospory and heterothally might have arisen together, the latter as a result of food stored in the enlarged spores. These spores might have in turn produced only female gametophytes, thus initiating heterothally. The spores with less food became pollen. Then after the female spore commenced to be retained, and the need for an ample food supply became less acute, it tended to decrease in size, and in many instances ultimately became actually smaller than its compliment, the pollen spore. As a necessary prelude to the seed habit, the particular species of *Archaeopteris* under consideration might have provided the heterothallic stage. The sporangium of *Archaeopteris,* however, in no manner portends a cupulate organ, but is more reminiscent of a psilophytic spore case in which numerous fertile telomes became clustered, with the clusters arranged along an axis which occasionally bore vegetative foliage along its lower portions. The problem of tracing a connection between the earliest seed plants and their cryptogamic forebearers is that of finding the oldest plant showing heterothally, a difficult quest because of the rarity of preserved gametophytes in fossil plants.

OVULE AND SEED

Opinions differ among paleobotanists as to whether the mature ovules of the pteridosperms and Cordaitales should be called seeds. Most investigators, including Scott and Seward, refer to them as seeds, although they admit the absence of an embryo, the one structure that is used to distinguish the seed from the ovule as the seed is defined in modern plants. Others (Benson and Emberger in particular) insist that in the absence of an embryo, the organ has not passed beyond the ovule stage and should be designated accordingly.

The matter could be dismissed as having no more than academic interest were it not for the concern some botanists express over it. Using the lack of an embryo as his criterion for classification, Emberger (19) has proposed the name "prephanerogams" for the pteridosperms and Cordaitales. They are, he says, not phanerogams but vascular cryptogams with ovules wrapped in open sporophylls. The female sporangia are monosporangiate, not many-spored, as in the majority of vascular cryptogams. The plants are therefore angiosporangiate vascular cryptogams, as opposed to all other vascular cryptogams which are gymnosporangiate. The pteridosperms and Cordaitales bear a relation to the vascular cryptogams that is similar to that which the angiosperms bear to the gymnosperms. These angiosporangiate vascular cryptogams are on their way to becoming phanerogams, which is the reason Emberger calls them prephanerogams.

Although Emberger is quite correct in assigning the pteridosperms and Cordaitales to an intermediate position between spore-bearing vascular plants and seed plants of the modern type, he commits the oft repeated error of attempting to define a major plant group on some single character that may be found to run through several genetically unrelated lesser groups. Such methods of classification, though convenient and simple, do not arrive at natural groupings. Lacking embryos, the pteridosperms and Cordaitales are both on approximately the same level of development, but they should not for this reason alone be placed within the same class or division, or whatever category Emberger intended the prephanerogams to constitute. The pteridosperms belong to the great fern-cycad plexus, whereas the Cordaitales belong near the base of the coniferous branch of the phylogenetic tree. Their position with respect to other plants may be compared with the pupils in one grade in a public school. Those pupils in the fourth grade, for example, are intermediate between the third and fifth grade pupils, but that does not indicate that they are all relatives. It simply means that, although they are all on approximately the same physical and intellectual level, each one has attained that stage of development independently of his fellows.

The present author does not believe that any genuine difference exists between the seedlike ovules of Paleozoic plants and the seeds of modern ones. The fact that the overwhelming majority of the ancient ovules are detached indicates that a state of maturity had been reached and that the shedding had occurred naturally at a particular stage in development. Furthermore it is quite evident that the ovules at this stage underwent a rest period, otherwise embryos and stages in seedling development would occasionally be found. Also they occur in too large numbers to permit us to assume that those shed without embryos were malformed. The normal form would be expected to occur in equal abundance at least. A brief lapse between shedding and germination seems essential to account for the presence of the hard durable integument that developed in most Paleozoic seeds. It would be difficult to account for this tissue if fertilization were always followed by immediate and uninterrupted growth of the new sporophyte, especially in the kind of environment that prevailed throughout the greater part of the Carboniferous period. The conclusion seems almost inescapable that in the pteridosperms and Cordaitales, embryo development was delayed until the termination of a rest period. During the evolution of the seed the rest period came first, and its inception was marked by the appearance of a durable integument. The integument could have served as a protection against water loss, or, as seems more probable, it prevented the intrusion of parasitic organisms. In modern plants the variations in the length of the rest period among different species probably has come about as a result of the interaction of environmental and physiological factors.

The numerous occurrences of pollen grains within the pollen chambers of fossil seeds furnishes ample proof that pollination had already occurred before shedding. There is also substantial evidence that fertilization had also taken place before fossilization because in some seeds, *Stephanospermum akenioides* for example, there are small openings in the pollen grains through which sperms probably emerged. In other seeds the upper part of the nucellus had been digested away so as to permit free access of the sperms to the archegonia which can sometimes be observed in well preserved material. With evidence that fertilization had already taken place before the ovules were fossilized, the young sporophyte stage had been initiated, and although there is no visible embryo there was nevertheless diploid tissue present. It therefore seems unduly dogmatic and arbitrary to exclude from the seed category these ripened ovules that occur in such copious quantities in the rocks. If a fully formed embryo must be present before an ovule can be called a seed, some of the modern cycads and orchids in which the ovules contain only rudimentary embryos at the time of shedding would have to be excluded from the seed plants. In the ancient plants all the essentials of a seed are present, the only difference between their seeds and most modern seeds being the relatively undeveloped embryos.

It may be that a new definition of a seed would satisfy the requirements of those who would still insist on an embryo of a given size before the ovule would rank as a seed. Although there are several possibilities, the following definition would certainly be adequate in the majority of cases: A seed is an ovule containing a fertilized egg, that is ready to be shed from the plant. Of course it may or may not be enclosed within a fruit, but in either case it is ready to part company with its parent.

Darrah (16, 17) has reported dicotyledonous embryos in cordaitean seeds from Iowa, but he has published no figures or detailed descriptions. Consequently it is impossible to judge whether he found embryos or merely saw the crumpled walls of the partially collapsed female gametophytes which might readily give a deceptive appearance of embryos. Reed (34) noted the presence in *Cardiocarpon affinis* of structures which at first sight suggested embryonic tissue but which, she decided upon further examination, were small rootlets which had invaded the endosperm.

RECENT WORK ON THE STRUCTURE OF PALEOZOIC SEEDS

In 1939 Reed (33) described four specimens of *Conostoma oblongum* found in a coal ball from Harrisburg, Illinois. Because of the small size of the seeds, serial sections could not be made, so the specimens were studied from smooth surfaces by reflected light. The surfaces were ground with fine corborundum powder, and at frequent intervals they were coated with Canada balsam and drawn with the aid of a camera lucida. When an especially desirable plane was revealed, the surface was permanently mounted and the grinding carried out on the reverse side until a thin section was prepared. This enabled her to give a detailed account of the "lagenostome" and pollen chamber.

In the same paper Reed described the structure of the large seed from Boonville, Indiana, which had been figured previously by Chamberlain (15, fig. 23) and subsequently by Stützer and Noé (40, fig. 79) as *Trigonocarpus*. When the specimen was cut, it was found that the exposed portion revealed the inner lining of the integument. The main part of the integument had been broken away from the base of the seed so that the original size could only be estimated. However, it must have been about seven cm. long and three cm. in diameter. The integument was more complete where the seed was still embedded within the coal ball, but preservation was rather poor. The nucellus and female gametophyte were indistinct, although the latter could be recognized as a shriveled structure occupying the central portion. She commented upon the absence of an embryo.

Miss Reed identified this seed as *Pachytesta gigantea,* a species named by Brongniart from the Stephanian of St. Étienne, in France. Hoskins and Cross (26) later made it the type of a new species, *Pachytesta Noei.* Reed transfers it from *Trigonocarpus* to *Pachytesta* on account of the thick integument. Just how thick the integument must be to distinguish *Pachytesta* from *Trigonocarpus* is not explained, but she says that the "three ridges", which were largely responsible for the original determination as *Trigonocarpus,* are not integument ribs but are the marks of sutures of the integument. Unfortunately the author does not figure the external surface of the seed or specify whether these ridges are any of those shown on Chamberlain's original figure. Three ridges do show on the exposed surface of the specimen where the outer portion of the integument is missing, but the exposed part is only half of the seed surface, and there are obviously six altogether. Mention is made of three valves in the rather poorly preserved integument which are separated by dehiscence lines, but whether these are the same as or bear any relation to the angles shown in Chamberlain's figure, the author does not explain.

In a later paper Reed (34) described specimens of *Cardiocarpon affinis* preserved in limy shale from the Cherokee and Des Moines series of Iowa. In the seeds small rootlets had invaded the endosperm. They appear to have entered through the micropyle in some instances but in others through cracks in the integument.

Kern and Andrews (1946), in a recent paper, have described three petrified seeds from the Des Moines series of Iowa. One of these is *Conostoma oblongum* which had previously been reported from North America by Krick (30) and by Reed (33). Another is a new species of *Rhabdospermum, R. spicatum.* It is a platyspermic seed 15 mm. broad by 12 mm. high. Its rather complex integument is four-layered with a double sarcotesta on the outside and two sclerotestal layers on the inside. The nucellus is free from the integument but is not well preserved. The pollen chamber resembles a shallow inverted cup. *Kamarospermum Leeanum* represents a new genus and species in which the peculiar feature is a basal chamber that probably lent bouyancy to the seed. It is a flattened seed, evidently cordaitean, about 12 mm. long, 11 mm. broad, and 3 mm. thick. The basal chamber is a feature resembling *Codonospermum,* from which it differs, however, in being platyspermic and in having a more complex integument with two sclerotic layers. The basal chamber is surrounded by the inner of the two. The micropyle shows two rather clearly defined regions, a massive proximal portion which is a continuation of the integument above the nucellar chamber, and a delicate distal part shaped like a flattened funnel. The pollen chamber is not preserved.

Two detailed papers on *Pachytesta* by Hoskins and Cross (25, 26) have recently appeared. The first one describes *Pachytesta vera,* a new seed, from Des Moines series. The seed is smooth, ovoid, and radially symmetrical, and has a maximum width of 3.5 cm. and a total length of 6.5 cm. It is a typical member of the Trigonocarpales. The integument is free from the nucellus except for the basal attachment, and the stony layer of the integument bears three prominent longitudinal ribs. Both the integument and the nucellus have a vascular supply. In the domelike distal end of the nucellus is the unspecialized pollen chamber in which were found numerous pollen grains and non-functional negaspores in tetrahedral groups. Eight of these tetrads were found, which they assumed to indicate the number of megaspore mother cells originally present. The individual non-functional spores measure 600–700 microns in diameter. The size of these bodies precludes the possibility of their having passed through the micropyle from the outside.

The broadly elliptical pollen grains in the pollen chamber measure about 45 × 63 microns. They are bilaterally symmetrical with an inflated peripheral bladder which appears to surround the central part except for the small distal area. They are of the type which when found separately are placed in the form genus *Florinites,* and which have been regarded as characteristic of Paleozoic gymnosperms of the *Cordaianthus* and *Walchianthus* types.

The non-functional megaspores probably represent the approximate size shown by these spores immediately after tetrad formation and before they had undergone any appreciable enlargement. They may therefore be assumed to indicate the size of the mature spore previous to the development of the prothallium. Since they are noticeably larger than the pollen grains, a true heterosporous condition can be said to exist in the plant of which *Pachytesta* vera is the seed.

In the second paper by Hoskins and Cross (26) the genus *Pachytesta* is revised, and the taxonomic problems which it presents are reviewed. The authors show that the *Pachytesta* type of seed is capable of producing different appearances, depending upon the extent of removal of the outer tissues before preservation, and they stress the difficulties in recognizing the original seed type when preserved in different ways. The authors point out that the name *Trigonocarpus* was originally given to three-ribbed casts showing no internal structure, but that later authors have extended the use of the name to seeds preserved as petrifactions and showing structure. They propose, therefore, that the name *Trigonocarpus* be restricted to casts of seeds which have three prominent longitudinal ribs and are identifiable only on superficial characters. They would then transfer to Brongniart's genus, *Pachytesta,* all seeds heretofore described as *Trigonocarpus* in which preservation allowed the internal structure to be observed. The genus *Pachytesta,* as they define it, would include the following seeds:

Pachytesta incrassata Brongniart
P. gigantea Brongniart ex Renault
P. vera Hoskins and Cross
P. Noei Hoskins and Cross
Trigonocarpus pussila Brongniart
T. shorensis Salisbury
T. olivaeforme Williamson

The seeds that Scott and Maslen had assigned to *Trigonocarpus Parkinsoni* are placed in *Pachytesta olivaeforme* along with *T. olivaeforme.* Although they make no mention of them, Hoskins and Cross would probably include under *P. olivaeforme* the well known figures of *T. Parkinsoni* by Scott (35, figs. 79–81) and by Seward (36, fig. 426).

In a large number, perhaps the majority, of instances, the principle of applying different names to seeds preserved in different ways is a sound one. It is usually the only approach to an orderly system of nomenclature. On the other hand, it is questionable whether this should be done as a matter of routine practice, and whether it is justified in this particular instance. In their emended diagnoses of *Trigonocarpus* and *Pachytesta* Hoskins and Cross include the tritartite character of the integument in both. The former is described as having a smooth surface "with three more or less prominent longitudinal ribs", and the latter reveals an "integument more or less divided into three approximately equal valves by longitudinal commissures". This anatomical feature is one whereby the *Trigonocarpus* form would be visible in the *Pachytesta* petrifaction, and the distinction is entirely the result of preservation. This being the case, there seems to be no good reason for applying separate generic names to them. *Trigonocarpus* is certainly just as appropriate as *Pachytesta* for seeds of the Trigonocarpales plexus with three prominent ribs or valves in the integument, and being the older of the two names, is the valid one. If, at any time, however, a seed should be found with the characteristic *Trigonocarpus* integument, but with other features not possessed by the Trigonocarpales, then a distinction of the kind Hoskins and Cross advocate would be justified. It also happens that seeds originally identical with *Trigonocarpus* are preserved in other forms (*Neuropterocarpus, Holcospermum* and *Carpolithus*), depending upon the degree of removal of the outer tissues. These preservation forms, however, do not alter the application of the names used for the more readily recognizable types.

It seems obvious, therefore, that the concept of *Trigonocarpus* could be broadened to encompass those anatomical features Hoskins and Cross include under *Pachytesta,* and that the genus could be extended to embrace both the three-ribbed casts and petrifactions when there is substantial evidence that they were both formed from the same kind of seed.

Loubière's work on the structure and classification of fossil seeds has been previously reviewed (4). His later papers have not been seen, but some of them have been summarized (19). Loubière (according to Emberger, p. 276) distinguishes three types of pteridospermous ovules; the Nertocaryales, in which the nucellus and integument are fused; the Mesocaryales, in which the nucellus and integument are free except at the base; and the Acrocaryales, based upon the single seed *Leptotesta,* which is briefly discussed in the next paragraph. This classification is apparently a substitute for an earlier scheme of Lagenostomales, Trigonospemales and Leptotestales proposed by him in 1930 (31). It is readily apparent that the Nertocaryales are equivalent to the Lagenostomales and the Mesocaryales to the Trigonocarpales.

In Loubière's Acrocaryales (Leptotestales) the nucellus is free from the integument except at the summit of the ovular cavity where it is attached by a ring of contractile tissue which pushes the nucellus up against the micropyle at the time of pollination. Loubière's description of *Leptotesta* in not entirely convincing, and Emberger (19, p. 277) gives a different interpretation of it. He believes the detached nucellus is the result of faulty preservation, and that normally it was joined to the integument, making the ovule a member of the Nertocaryales.

Emberger (19, p. 273) gives a brief description of *Coleospermum,* an ovule described by Loubière, but whose original account has not been seen by the present author. In this ovule there is a stigma-shaped excrescence of the nucellus just beneath the micropyle, and directly under this is a chamber which has been formed, apparently by digestion, between the epidermis of the nucellus and the embryo sac membrane. The drawings show two archegonia in the tissue of the embryo sac. The microspores fell into the nucellar chamber where they germinated and produced minute male gametophytes. From these were released ovoid-pyriform sperms which were probably non-motile. How the pollen reached the chamber beneath the stigma-like outgrowth of the nucellus is not explained.

Steidtmann (37) has recently added a few details to the description of *Rotodontiospermum illinoiense,* from the McLeansboro of Illinois. In the original description of the seed the apical part was not completely treated. More recently discovered specimens show an apical beak about 11 mm. long with the inner flesh extending into it for about four mm. In the top of the nucellus there is an indentation which is believed to be the pollen chamber, although it is not surmounted with the beaklike tube described for petrified specimens of *Trigonocarpus*. No pollen or spores were found within the pollen chamber.

Gothan (22a) has reported *Trigonocarpus*-like seeds and pollen-bearing organs of the *Boulaya* type in such intimate association with foliage of *Lonchopteris rugosa* that attachment is almost a matter of certainty. The seeds, which are placed in a new genus and species *Dictyotesta lonchopteroides,* are very incompletely preserved compressions, and the characteristic feature is a fine meshwork in the integument in the perimicropylar region. The seeds were loosely enclosed in hulls of partly separated fibrous bracts.

RESUME AND CONCLUSIONS

The substance of the science of paleobotany still consists largely of "loose ends", regardless of the achievements wrought during the last three decades. This situation is nowhere more in evidence than when dealing with fossil seeds of Paleozoic age. Few noteworthy instances of the attachment of seeds to vegetative parts have been reported within recent years, and the relatively small number of examples of attachment listed by Halle (23) constitutes the main body of our knowledge of seeds in relation to the plants on which they grew. As stated before, no actual seeds have been found in the Devonian. The supposition that seed plants existed then stems from indirect evidence. The seeds reported by Dawson (18, p. 82) from eastern Canada have been known for many years to have come from rocks of Pennsylvanian age.

It is believed that the terminally borne cupulate seed found in the early Lower Carboniferous is a primitive organ which may have been derived from the terminal spore case of the Psilophytales. The classical theory that the seed evolved from a leaf-borne sporangium has no support in the fossil record. Several ferns, which are scarcely beyond the psilophytic stage, have been found in the Middle Devonian. In *Protopteridium,* the only one for which the fructifications are known, the sporangia are on the tips of forked branchlets, as in Rhynia. Some of the purely vegetative branches are flattened and resemble pinnules or simple leaflets.

The whole branch system resembles a frond. In this plant the foliar organs are evidently sterilized and flattened branchlet systems. The evidence furnished by this plant is that the fructification came first and that the leaf followed. In the Upper Devonian genus *Archaeopteris* the differentiation between fertile and vegetative systems is carried a step farther. Whole pinnae, or the forward portions of pinnae, are fertile. The oval sporangia are terminal and single on small slender stalks borne in regularly spaced groups on opposite sides of the pinna rachis. Some of the sporangial stalks are forked. Usually there is an abrupt transition between fertile and sterile portions, but frequently some of the stalks in a cluster lack sporangia and are joined by a lamina, so that the cluster intergrades with a vegetative pinnule. This transition indicates that in *Archaeopteris* the vegetative pinnule was a potential sporangial cluster from which the sporangia were suppressed and the stalks joined with photosynthetic tissue. The leaves, therefore, were derived from fertile structures.

Because of the relative simplicity of all known Paleozoic seeds and the lack of well developed embryos in them, some investigators refuse to recognize them as seeds, but insist that they are ovules only one step in advance of the cryptogamic sporangium. Whether or not these organs are seeds is merely a matter of definition, and the intermediate status of the pteridosperms and Cordaitales in the plant kingdom is not altered.

There is no proof that heterospory, as the term has been historically defined, was ever an essential factor in seed plant evolution. Difference in spore size has probably come about independently in different plants, but it has never characterized or dominated seed plants. Heterothally, on the other hand, was already in evidence at the initiation of the seed habit. The current notion that heterospory dominates seed plants is the result, as Thomson explains, of a misunderstanding of Hofmeister's writings and of the prevalent but misleading habit of comparing the size of the completely formed embryo sac with that of the pollen grain. Since the spores of seed plants, although not necessarily differing in size, produce two kinds of gametophytes, Thomson's proposal that the pollen spore be called an "androspore" and the seed spore a "gynospore" is a pertinent one. These names could appropriately replace "microspore" and "macro-" or "megaspore" in botanical literature dealing with seed plants. Not only did the two kinds of spores in seed plants probably evolve from isospores of early cryptogams, but the fructifications bearing the two are obviously homologous.

The different ways a seed may be preserved greatly complicate the whole matter of seed nomenclature. It is necessary that different generic names be maintained for different forms of preservation. In general, the name originally given to some particular form should not be used for a seed preserved in a different way, but if there are obvious anatomical characters displayed which reveal their identity, one of the names may be rejected.

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FACTORS EFFECTING THE DISTRIBUTION OF DIATOMS

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INTRODUCTION

The distribution of diatoms has attracted the attention and consideration of diatomists almost from the beginning of their study. At first it was simply the comparing and contrasting of floras in various parts of the world without any attempt to correlate or interpret their findings. Ehrenberg, from 1843 until his "Microgeologie", published in 1854, brought together a great amount of facts on this subject. In his "Microgeologie" he charted in table form the distribution of diatoms in various continents.

The next great work to bring together information on the distribution of diatoms was that of De-Toni, "Sylloge Algarum". In this work, under each species considered, he gives the known distribution of that species. However, no attempt was made to correlate this information. P. T. Cleve (36) seems to have been the first to make a careful study of diatom distribution. He states that although many species are cosmopolitan, occurring in all parts of the world, there are, on the other hand, many species, genera and groups which occur in only certain seas and climates. Likewise in fresh-water species we find characteristic species of certain regions.

Since the time of Cleve many others have commented on the distribution of species of a given region. It is from these works that the facts were brought together to make possible this paper. Between 350 and 400 papers were read, but only those from which information was used are cited.

When one thinks of diatoms he almost automatically divides them into marine and fresh-water species. This, in general, is a very good division to use in the consideration of diatom distribution, for the factors which act as barriers to distribution are very different in the two groups. It is under these headings, therefore, and the subheadings of ecological and geographic factors that the distribution of diatoms will here be considered.

MARINE DIATOMS

Ecological Factors

General. In considering the ecological distribution of marine diatoms, one thinks at first of littoral species which grow attached to or closely associated with the bottom, and of planktonic species which spend all or at least the vegetative part of their lives afloat. The truly oceanic planktonic species not only spend their vegetative life afloat but also reproduce in the open sea. The neritic planktonic species, however, are afloat during their vegetative stage, but their spores fall to the bottom and rest there during unfavorable conditions.

Thus it is evident that the ability to float is essential for the existence of the species in planktonic form. There are various ways in which the structure of the diatoms has been modified for this function.

Many of them have various types of processes to produce more surface area. Gran (66) classifies them into the following types: the bladder type, *e.g., Coscinodiscus,* in which the cell is large and the protoplasm occupies a thin layer around the outside, while the center is filled with a thin fluid of about the same specific gravity as water; the ribbon type, *e.g., Fragilaria,* in which the diatom is flattened and thus has a broad surface in one plane; and the hair type, *e.g., Rhizosolenia,* in which the cells are greatly prolonged in one direction. Cells of this last type produce a great deal of friction when horizontal but sink rather rapidly when perpendicular. In the branching type, *e.g., Chaetoceros,* the cell surface is enlarged by various types of hair-shaped or lamelliform outgrowths. Ostwald (66) found that species in regions where the temperature is very variable often have summer and winter forms, the summer forms having more surface area because the viscosity of the water is less and more surface is required in order to keep afloat. For example, the two forms of *Rhizosolenia, hebetata* and *semispina,* were originally thought to be two distinct species. *Rhizosolenia hebetata,* the Arctic form, has a thick wall and attenuated ends, while *R. semispina,* the Atlantic form, has thin walls and hair-like ends.

Allen (12) finds that the cells of diatoms which spend their life afloat have thinner walls than those of sedimentary forms, and many of the former exhibit various types of protuberances which delay sinking.

Riley (143) has found that under unfavorable conditions of light or nutrients diatoms may keep afloat by passing into a state of senescence. When in this condition the organic content and thus the weight of the cell is decreased and the ends of the cells often become curved. In this state they seem to remain viable for years.

Production of oil and gas within the cell also helps to keep it afloat (79, 12). Schreiber has found that in *Biddulphia mobiliensis* oil is stored under the influence of strong light. Gran, in a personal communication to Braarud (22), states that oil seems to accumulate late in the vegetative stage and that he interprets this as being due to a deficiency of nitrogen. Riley (141) found that *Coscinodiscus* sank faster in April than in March. This was due to the fact that the diatom was using up the stored oil as photosynthesis slowed down, which was due to depletion of nutrients.

Salinity, Temperature and Viscosity. Another important factor in determining whether diatoms float is the viscosity of the water in which they live (22, 55). Temperature is the main factor which influences the viscosity of sea water. Ostwald has found that at 25° C. sea water offers only one-half the resistance to the sinking of diatoms as at freezing. Perhaps one of the reasons why diatoms seem to have their best development at certain specific depths is that at that depth the viscosity is sufficient to keep them afloat (7). The tendency of warm waters to exhibit better development of diatoms at lower levels than cold waters may be due in part to the difference in viscosity of warm and cold water (140).

The fact that diatom species are specific as to their salt requirements has long been recognized. The early division of species into fresh, brackish and salt water forms was based on this fact. Thus the association of certain species into a flora, and the delimiting of that flora to a certain region, is often in part due to a difference in salinity. For example, Gran and Hjort (83) believe that the difference of diatom flora in Lemfjord and the North Sea is largely due to a difference in salinity between Lemfjord and the North Sea. The characteristic floras of ocean currents at a given time seem to be at least in part due to different salinity requirements. Gran (67) noted at Romsdalen that a definite diatom succession occurs. In the latter part of March *Skeletonema costatum* and *Chaetoceros socialis* developed, but soon *Chaetoceros debile* became dominant. *Chaetoceros debile* is replaced in turn by *C. constrictus* and *C. compressus.* In July *Rhizosolenia alata* became the dominant species. Such a succession of dominants is probably due to the special requirements of each species for temperature and salinity, but the abundance of the plankton is dependent on the nutrients present. Braarud (22) also states that salinity, pH and other factors affect the kind of species that may be abundant rather than the total amount of plankton.

Temperature and salinity may also affect the extent of an ocean current and thus indirectly the distribution of diatoms. Nathansohn (120) states that temperature and salinity of the water are important factors in determining the extent of the Atlantic and polar waters. The Atlantic water is saltier than the polar water. In summer when the Atlantic waters become very warm they become lighter than the polar waters and thus spread farther north, whereas in winter when the Atlantic waters become cool, the polar waters, having less salinity, are lighter and spread farther south.

Temperature, in addition to affecting the viscosity of sea water, has many other effects, both direct and indirect, on diatoms. It seems to influence both the kind and abundance of species. Brandt (23) was the first to show the effect of temperature on the nutrient content of the water. In cold water nitrates are allowed to accumulate, due to the fact that the low temperature hinders the activity of denitrifying bacteria. The abundance of diatoms in the polar seas is partially due to this effect. Conversely the low nitrate content of warm water is due to the great activity of denitrifying bacteria. Thus the surface waters are not rich enough to support an abundant flora. However, as pointed out by Riley and Allen (140, 12), there may be an accumulation of diatoms at lower depths where the water is cooler. Also the higher the temperature of the water the less the change of bicarbonates to carbonates, and the formation of carbonic acid is definitely depressed (120). Mann (113) believes that the abundant growth of diatoms at each of the poles is in part due to the large amount of carbonic acid held there in solution, which is made possible by the low temperature of the water.

Temperature seems to affect the germination of spores. Gran (65) believes that one of the reasons for the abundance of diatoms in cold water is that the temperature favorable to their germination is lower than that necessary to stimulate bacterial activity. One reason why diatom blooms usually occur earlier in the spring in coastal waters than in the ocean, is that the arctic or northern neritic species start to develop at a lower temperature than the Atlantic oceanic forms. Again (22) the occurrence of a rich diatom flora at the edge of melting ice is partially due to the fact that the melting ice contributes spores of species which can germinate at a low temperature. Temperature is also one of the main factors which cause different species to occur at different latitudes and in the various currents. The reason that a current at a given time of year can be characterized by a specific diatom flora is that it has a given salinity, dissolved gas content and temperature which are favorable for their development. For instance, Schreiber (146) has found that *Biddulphia aurita* has a lower temperature optimum than *B. senensis*. Thus in the North Sea *B. aurita* is abundant in winter and *B. senensis* is abundant in summer. Allen (8) notes that *Skeletonema costatum* prefers cool waters and is found either at northern stations or in the cool part of the year. On the other hand, *Chaetoceros curvisetum* requires a temperature of 18°–20° C. for best development (67). Many other examples of this sort could be given.

The plankton of the tropical Atlantic shows a greater diversity of species than that of polar regions, though the abundance of individuals is not so great. Allen (6) finds support for the statement that the higher the latitude the richer the plankton, though, of course, exceptions do exist and there is no definite evidence that high latitudes are more productive year by year per thousand square miles than low ones (Allen, personal communication).

Light. The effects of heat can not be divorced from those of light, and therefore these two factors which affect diatom development and growth are somewhat integrated. Germination of diatom spores seems to be affected by light (65). Kreps and Verjbinshaja (104), in their studies of the productivity of diatoms in the Barents Sea, noted that at the time of spring flowering of diatoms in the coastal water and in water near the arctic ice, there was a meagre diatom flora in branches of the North Cape current of the Atlantic waters, though it is rich in nutritive materials. They believed that perhaps this is due to the fact that though light conditions are ideal for development of spores of arctic species, they are not right for the development of spores of species carried by the Atlantic current.

As light is necessary for photosynthesis, it follows that for best growth, diatoms must remain in the photosynthetic zone. This zone varies not only with geographic latitude and season but also with the transparency of the surface layers and the density of the plankton (69). As with other plants too much or too little light may be harmful to this process. Not only the intensity but also the duration of light is important. The meagreness of diatom growth has in some cases been attributed to lack of sufficient light which may be due to turbulence of the water or lack of solar radiation. Riley (141) found that at George's Bank in the Gulf of Maine vertical turbulence and solar radiation were the chief limiting factors on plankton growth throughout the late autumn, winter and early spring. Gran and Braarud (70) attributed the small diatom growth in the Bay of Fundy and along the New Brunswick coast to lack of sufficient light due to turbulence. They state that in order for a diatom to survive it must be exposed to light at least one-fifth of the time. The idea held by some that the intensity of radiation during winter in the north temperate zone is not sufficient for abundant diatom growth is somewhat refuted by the results of research (115, 141) wherein it was noted that not only is there sufficient light for diatom growth in mid-winter, but that on some days it may be strong enough to inhibit growth.

The depth at which diatoms will grow is in part due to the amount of radiation which they can receive. Marshall and Orr (115) suggest that at the deeper levels day length is an important consideration. Allen (8) noted that certain species of diatoms reach their best development at various specific depths. For example, at the surface, where there is the greatest amount of radiation and substances derived from the atmosphere, *Chaetoceros* sp. reached its best development. *Hemiaulus hauckei* was most abundant in the upper ten meters. *Chaetoceros compressus* was richest at 20 meters. *Chaetoceros scolopendra* and *Thalassiothrix nitzschioides* were found only below 20 meters. In southern California waters the greatest abundance of diatoms occurs between 20 and 35 meters (51). Karsten (90) found the best development of diatoms in the

Antarctic between 20 and 80 meters, while in the Indian Ocean the best development was between 80 and 100 meters. Riley also states that in the north the greatest diatom development was between the surface and a depth of 100 meters. In the south the greatest abundance was between 100 and 400 meters. Riley (140) found the optimum light penetration in northern water five meters, in the Sargasso Sea 80 meters. Though light undoubtedly is an important factor in determining the depth at which diatoms may live, it must be remembered that nutrients, viscosity of water, *etc.*, also influence their ability to grow at various depths.

For the best development of diatoms Nathansohn (120) reports that both light and nutrients must be correct. The beginning of spring flowering is considered by some to be conditioned by light. Marshall and Orr (115) believe that the critical cause of the beginning of the spring maximum is the total amount of light which in turn is dependent on day length and brightness. Atkins (15) observed at Plymouth that the spring diatom outbursts occurred before the vernal equinox, when the day length was 10 to 12 hours and about three hours of sunshine were present. The variation of the time of outbreak seemed to be brought about by variation in sunshine. Riley (142) reached the conclusion that a balance between the effects of vertical turbulence and the increase of vernal radiation determines the beginning of spring diatom flowering. According to several authors (77), the duration of the autumn outburst is controlled by the amount of light available. Davidson and Huntsman (50) state that the reason for the negligible amount of diatom growth in winter is the small amount of light and low temperature. The abundant growth of diatoms in the polar regions is due in part to the abundance of continuous light during a long portion of the year (113).

Nutrients. Since the time of Brandt (23) the importance of the nutrient content of the water to diatom growth has been recognized. He was the first one to suggest that Leibig's law for land plants might apply to diatoms. The law states that any nutritional requirement if not present in sufficient amounts may act as a limiting factor of the assimilation of other nutrient substances. He and Hensen (80) pointed out that plankton production is closely correlated with the amount of nitrates present. A deficiency of nitrogen may act as a limiting factor. Cooper (46) states that the most important source of nitrogen for diatom growth is the nitrate form. Gran (68, 146) and others have suggested that one of the important factors which makes possible the spring bloom of diatoms is the abundance of nitrates in the water. During winter when the temperature of the water is too low for much bacterial activity nitrates are allowed to accumulate.

Phosphorus also seems to be a very important element for diatom growth (65). Hentschel (81) found on the west coast of Africa that the regions of diatom and phosphorus abundance are very similar. Likewise Gunther (72) found on the western coast of South America that in waters rich in phosphorus the diatoms are particularly well developed. Phosphorus may also be a limiting factor in diatom growth. Marshall and Orr (115) noted that when the PO_4 was used up the diatom maximum disappeared. It, as nitrogen, may be responsible for diatom succession (70). Lillick (109), from her study of diatoms in the Woods Hole region, concluded that NO_3 is more critical for phytoplankton production than PO_4. One of the causes for a succession of different species in a flora seems to be the various requirements for these mineral substances. Gran and Braarud (70) found in the Gulf of Maine that when the water was rich in nutrients a *Thalassiosira* plankton was dominant. As the NO_3 and PO_4 were used up, species, including *Rhizosolenia alata,* which can live on less nutrients came in. Thus depending on the nutrient supply, the species of a flora may vary.

The importance of other mineral elements in diatom growth has also been recorded. Braarud (22) states that the calcium content as well as the pH and salinity affect the kind of species present rather than the abundance. Silicon is also important to good diatom growth (65, 117). Hendey (79) believes that one of the reasons for the thin walls of diatoms in tropical waters is the low silica content of the water. Iron has also been found to act as a limiting factor in diatom growth (69). Harvey (75) found that diatoms were very dependent on iron for rapid growth and that lack of iron checked growth. Ferric hydroxide or phosphate can be used by diatoms either in colloidal form or as larger particles. The iron hydroxide is absorbed on the surface of the diatoms and thus is in contact with an interface where solution and subsequent passage into the cell are probable. The amount of carbonic acid held in solution may also be a factor affecting the abundance of diatoms.

Certain substances have been found to act as stimulants to diatom growth. Harvey (74) found that addition of one milligram of citrate of iron to a cubic meter of enriched sea water doubled the growth rate of the diatoms. Addition of one part per million of manganese also stimulated their growth (76). Gran (68) states that the much richer development of diatoms in coastal waters can not completely be explained by NO_3, PO_4, light, temperature and salinity alone. He believes that perhaps the fresh waters from land carry humic acid and organic compounds which stimulate growth. Harvey (76) found that extracts of soil greatly stimulated diatom growth. Extracts of *Fucus* (76) and of *Ulva* (3, 76) were also recorded as serving the same purpose.

From the above studies it is clear that the kind and abundance of nutrient salts are very important to diatom growth. Nutrients in the sea are received by drainage from land or are brought to the surface from deep water. Thus the regions of most abundant diatom growth are in the coastal waters where drainage from land occurs or where upwelling is taking place (51, 67, 81, 72). It is also true that rich diatom production occurs just in front of melting ice. Formerly it was supposed that it was some component or components of the melting ice water that stimulated growth. Braarud (22) states that the reason why phytoplankton is often abundant at the edge of melting ice is that the turbulence in the water caused by the melting ice brings up nutrients from the lower layer. The melting ice contributes spores but is not rich in nutrients.

The supply of nutrients is so closely correlated with diatom abundance that the periodicity of diatom blooms or richness can be forecast from the type of nutrient supply. Gran concludes that in northern Europe the plankton development may be any one of three types. When the nutrients are continually augmented by a supply from land or vertical currents, a diatom flora may continue throughout the year, unless light is a limiting factor in winter. If the nutrient supply is intermittent, the diatom maximum and minimum will alternate. In some cases there is little or no supply of nutrients derived from land, and vertical circulation is possible only early in the spring. Such conditions produce only one diatom "bloom", and that early in the spring.

Other factors. One other factor of importance which may limit diatom abundance is the feeding of plankton animals (65, 77). As diatoms increase by geometric progression, intensive feeding when the diatoms are just beginning to increase may greatly limit the bloom.

Other factors affecting successful growth of diatoms include excessive turbulence. Such a factor may hinder the ability of diatoms to float or may cause the water to become so muddy that insufficient light is available for photosynthesis. Still other factors, singly or in combination, such as exhaustion of nutrients, intolerable accumulation of wastes, epidemics of disease or attacks of predatory organisms, may also limit diatom growth (5).

Summary. From these various studies it is evident that much is yet to be learned concerning the chemical and physical factors which determine diatom growth and productivity. Once these are understood the phytoplankton will be more useful in helping to interpret the biodynamics of the ocean.

Geographic Factors

Northern Europe. The first seas to be studied intensively with respect to geographic distribution of diatoms were those around northern Europe. Workers were then led to a consideration of the North Atlantic and the Arctic Sea. Cleve (33) found that many forms which occurred frequently in the Arctic Sea were not in the tropics, and that some of the species which are nearly always in the middle and southern Atlantic were absent. Cleve, in his studies of the diatoms of Franz Josef Land, found that 55% were in Spitzbergen and only 26% in Lappland. No species of the genera *Gyrosigma, Pleurosigma, Cocconeis, Epithemia, Cymatopleura, Surirella* and *Campylodiscus,* which are so common on the continent of Europe, were on Franz Josef Land. The diatoms of Franz Josef Land (71) were in part more similar to the fossil diatoms in the polar slate of Simbirsk and in the "cementstein" and "moleren" of Jutland than to those of the Arctic Sea. However, the diatoms on the underside of the ice blocks on the west coast of Novaja Semlja were identical with those described by Cleve (34) from northern Siberia, but some of the most characteristic forms were missing. A study of diatoms gathered on the mud on an ice floe 48 miles south of Bell Isle (38) showed that the mud probably was derived from the Kara Sea, though some of the marine species were the same as those on drifting ice at Cape Wankarema.

A much greater similarity seems to exist between the floras of Cape Wankarema and the east side of Greenland (35, 64). The latter is characteristic of floating ice. Therefore there must exist a communication between the Sea of Siberia and the sea off the coast of Greenland. Likewise in the study of dust from drift ice north of Jan Mayen Land, Cleve (40) found that the marine diatoms were the same as those at Cape Wankarema. The fresh-water forms were of more local origin, being probably blown in by wind from land.

Similar conclusions were reached by Gran (64) from his studies of collections at Cape Wankarema, east coast of Greenland, Cape Eglinton, and some of the collections of Nansen from the Arctic Ocean. He also noted the difference between the diatoms of these collections and those of collections from Novaja Semlja, Karajak Fjord and the Barents Sea. These findings seemed to indicate not only that a polar current existed across the Arctic but also the direction of the current.

The influence of the polar sea is also seen in the plankton of Baffins Bay and Davis Strait, the latter of which is derived in part from the North Atlantic (37). Cleve found in this plankton an abundance of *Thalassiosira nordenskioldii* which characterizes the polar sea, and also in the Labrador current many species which had been noted on ice at Cape Wankarema, on ice flakes between Novaja Semlja and Franz Josef Land, and on ice flakes that drift along the east coast of Greenland. The Atlantic influence was apparent by the presence of *Chaetoceros atlanticus* and *Thalassiothrix longissima* which were abundant in the North Atlantic. Cleve believed that these findings indicated that the ice flakes drifted from the Bering Straits to the north of Greenland where they divided, part going to the east coast of Greenland and part to the Labrador current. Gran's (63) findings seem to support this theory.

As a result of his various studies Cleve came to the conclusion that each ocean current is characterized by a specific diatom flora. Thus the diatoms in a current would indicate the source of the water in that current. In 1899 he published the following list of the various plankton types of the North Atlantic Ocean:

a). The desmoplankton is characteristic of the warmest part of the Atlantic where the temperature of the water is 20°–28° C. Many of the species in this plankton are found in the Indian Ocean.

Some of the characteristic ones are *Chaetoceros coarctatus, C. tetrastichon* and *Climacodium biconcavum.*

b). The styliplankton surrounds the desmoplankton, in a temperature varying from 10° to 20° C. and a salinity of 35 p.p.m. It extends from the Caribbean Sea to Bermuda. The current bearing this plankton broadens out at about 40° N. and curves toward Europe and Africa: a part of it swings south to the Canary Islands and Cape Verde; a second part goes north, approaching in summer the Faeroes Channel and in autumn Spitzbergen. The second part sends off two branches, one through the English Channel into the German Ocean (didymus plankton), the other around Scotland into the North Sea (triposplankton). The temperature favorable to development of didymus plankton is 8°–17° C. and the salinity 32–33 p.p.m. The triposplankton prefers a temperature of 5°–14° C. and a salinity of 34 p.p.m. The styliplankton is composed of a large variety of species, some being brought in from the north and some from the south. Some species are found only in certain regions.

c). The chaetoplankton is confined to the northern and western Atlantic only in the spring. From March to June it may be traced from latitude 40° N., longitude 70° W., to the Newfoundland Banks and to the south of Iceland across to the Faeroes Channel and into the North Sea where it replaces the triposplankton. It disappears from the North Sea in summer, being replaced by the styliplankton. In July and August the chaetoplankton is found around Spitzbergen. When the chaetoplankton touches a coast, that of Iceland, for example, it sweeps away neritic plankton and spreads it along the coasts of Scotland and Scandinavia where it enters the fjords. Thus many species of northern origin may remain imprisoned during the summer in the fjords, especially in the deeper water. The chaetoplankton is found in a temperature of 5°–9° C. and in a salinity of 35 p.p.m. Some of the important diatoms which characterize this plankton are *Chaetoceros decipiens, C. constrictus, C. borealis, C. criophilus.*

d). The trichoplankton is confined in summer to the western and arctic Atlantic, in a temperature of 6°–12° C. and a salinity of 34 p.p.m. In winter it may spread to Scandinavia. Some of the species characteristic of this plankton were gathered from the Bering Sea on the Vega Expedition (34). Cleve questioned whether these species spread from the North Pacific to the North Atlantic or *vice versa.* In the winter of 1897–1898 some of the characteristic species of this plankton were found south of the Azores. Cleve believed that they were picked up from the Labrador current by the Gulf stream and thus conveyed southward. Some of the more characteristic species of this plankton are *Chaetoceros atlanticus, Coscinodiscus oculus iridis, Rhizosolenia semispina, Thalassiosira gravida, Thalassiothrix longissima* and *Rhizosolenia obtusa.*

e). The northern neritic plankton is found in winter along the coast of Iceland, in the Skagerack and in the fjords of Norway and Sweden. In summer it is slowly replaced by the triposplankton. The northern neritic plankton invades the coasts of Scotland and Scandinavia twice a year, in the spring with the chaetoplankton and in the autumn with the trichoplankton. The temperature at which this plankton grows is 4°–7° C. and the salinity 32–33 p.p.m. The characteristic species are *Asterionella spathulifera, Biddulphia aurita, Chaetoceros debilis, C. diadema, C. scolopendra, C. tires, Coscinodiscus polychordus, Leptocylindrus danicus, Skeletonema costata, Thalassiosira gelatinosa, Thalassiothrix frauenfeldii.*

f). The siraplankton has been found on the coast of Greenland, in Baffins Bay and in the Arctic Ocean. It is the plankton of water with melting ice drift. As it touches the trichoplankton it becomes mixed with it, so it is sometimes hard to tell the two apart. The water of the siraplankton has a lower temperature and less salinity than that of the trichoplankton. The characteristic species are *Thalassiosira nordenskioldii, Fragilaria oceanica, Lauderia fragilis, Chaetoceros furcellatus* and *C. socialis.*

g). The arctic neritic plankton is composed of a mixture of siraplankton and neritic forms found along the coast of Greenland. Some of the more characteristic species are *Amphiprora hyperbora, Achnanthes taeneata, Chaetocerros septentrionalis, Coscinodiscus bioculatus, C. hyalinus, Eucampia greenlandica, Fragilaria cylindrus, Navicula septentrionalis, Nitzschia frigida,* and *Pleurosigma stuxbergii.*

These studies led Cleve to believe that in summer the southern currents with their characteristic plankton extend farther north, while in winter the northern currents extend farther south. These and subsequent investigations of the North Atlantic and seas about northern Europe caused him (41) to be of the opinion that the shifting of plankton in a given region is due to a shifting of the various currents to which the respective plankton floras belong.

Though this theory was accepted by many, Hjort and Gran (83) believe that the idea supported by Cleve, Aurivillius, Petersen, Ekman and others, to the effect that the origin of a current can be determined by the type of plankton in it, is not correct. They point out that diatoms are very sensitive to changes in hydrographic conditions, such as temperature, salinity, *etc.*, and that an ocean current changes in these respects according to the season of the year and the direction of its flow. Therefore it would be impossible for the same diatom to dominate the plankton over a long distance. It is more probable that certain specific diatoms dominate the plankton of a given current at certain places and times of year. At any one time the various currents could be recognized by the plankton they support, for different currents would not have the same hydrographic conditions at the same time, and thus they would support different flora. Furthermore, the change of dominant species at a given locality is due to a change in the hydrographic conditions which make the environment more favorable for the development of species which hitherto under less favorable conditions have existed as resting spores. Hjort and Gran thus contradict the theory of Cleve that such changes are due to shifts in currents. They believe that species of diatoms can be used to tell the origin or direction of a current only when hydrographic conditions remain constant within relatively short distances, and that the abundance of a certain species indicates similarity of ecological conditions rather than a common origin of the water which contains them.

Gran (65) recognizes various plankton types much as Cleve did, but would classify them according to the type of condition in which they are found. He recognizes an arctic plankton, a boreal plankton and a temperate plankton, and subdivides each into oceanic and neritic forms. Cleve (44) states that his styliplankton corresponds to Gran's temperate Atlantic oceanic plankton, his didymosplankton to Gran's temperate Atlantic neritic plankton, his trichoplankton to Gran's boreal oceanic plankton, and his siraplankton to Gran's boreal neritic plankton.

Gran (65) further points out that currents are characterized by certain forms occurring in great abundance at specific times of the year. It is these blooms at definite times that characterize a current. At a given time the limits of a stream in a certain region can be determined by the dominance of characteristic species. In a region where there is a mixture of water currents one can tell something as to their origin by determining their plankton, but one can not state that just because an arctic diatom is present in a stream, there has been a mixture of arctic water.

Cleve (43) criticises Gran's theory that a diatom may persist in a given locality during unfavorable conditions by the formation of resting spores which drop to the bottom and become imbedded in the mud. His chief objections are three in number. First, no one as yet has germinated diatoms from bottom mud. Second, spores might drop to the bottom in a sheltered bay, but this would not be possible in rough water. Third, it is true that spores which have been found have mostly been of neritic species, but it is equally true there are many neritic species whose spores have never been found.

He (43) points out that Gran admits that certain species drift with currents. As an example he states that in February the usual littoral species, *Paralia sulcata,* was scattered over the whole of the North Sea. The very compact *Aulacodiscus argus* was found midway between Portugal and the Azores, seven degrees from the coast. The neritic forms, *Stephanopyxis turris* and *Eucampia zodiacus,* were midway between the Azores, the European coastal bank and Newfoundland.

In 1903 Cleve again stated that each current carries its own indigenous flora. There are in each current some species which are able to adapt themselves to changing conditions. Thus when two currents meet and their waters become mixed such species will spread. For example, *Coscinodiscus oculus iridis,* which belongs to the Arctic current in the western Atlantic, will establish itself in the eastern Atlantic west of Norway, when in winter and early spring the current expands eastward and mixes with the water of the temperate Atlantic.

The opinion held today by many diatom students is that diatom floras are characteristic of various currents because of differences in hydrographic conditions. At a given time probably no two currents would have the same hydrographic conditions, and therefore their diatom floras would be different. Thus at any one time the currents may be recognized by their floras. The theories that various currents invade a given region at various times of the year and thus produce a succession of floras, and that the origins of the water of a current can be determined by the species of diatoms present, need further verification. Nathansohn has, however, pointed out that the extent of the northern and southern currents depends on the season of the year. In winter the northern water, being less saline, tends to extend farther south than in summer, for in summer the southern water, though saltier, because of rise in temperature, becomes lighter than the northern water, and thus the southern currents at that season extend farther north than in winter.

Northwestern Atlantic. In the western Atlantic the main studies of diatoms have been made in the Gulf of Maine and its vicinity (19, 70, 108, 109, 150). The various types of plankton which may be found in this region during a year are arctic neritic, boreal oceanic, boreal neritic, temperate oceanic and temperate neritic.

The spring bloom starts from March to late April, depending on the locality. The type of species which dominate seems to vary somewhat over a period of years. Bigelow (19) found in March that in open waters *Coscinodiscus asteromphalus* was dominant, with the oceanic species, *Chaetoceros decipiens, C. atlanticum* and *C. criophilum,* universally distributed in the Gulf, while in the coastal waters, species of the genera *Thalassiosira* and *Chaetoceros* were the most characteristic members of the diatom flora. Likewise in 1935 (70) the more common neritic species were found to belong to these two last named genera: *Thalassiosira nordenskioldii, Chaetoceros debilis, C. compressus* and *C. diadema.* In addition, *Porosira glacialis* was also common. In 1941 Sears found at George's Bank that the dominant species were *Chaetoceros debilis, C. decipiens* and *Thalassiosira nordenskioldii.*

The summer flora is divided into three parts: early, middle and late. The early flora has as its dominant members *Leptocylindrus minimus* and a small form of *Thalassiosira* at George's Bank, *Chaetoceros debilis* in Passamaquoddy Bay, and *Chaetoceros* sp., *Corethron hystrix, Guinardia flaccida, Leptocylindrus danicus* and *Nitzschia seriata* at Woods Hole.

In mid-summer there was a flowering of *Guinardia, Thalassiothrix* and *Rhizosolenia* on George's Bank (150). Elsewhere in The Gulf of Maine and in the Bay of Fundy *Asterionella japonica, Chaetoceros constrictus* and *Skeletonema costatum* were the main species.

In late summer in the Woods Hole region (109) a temperate flora consisting mainly of *Rhizosolenia calcar-avis, R. alata, R. setigera* and *Thalassionema nitzschioides* was found. At Martha's Vineyard *Rhizosolenia* was the main genus. The late summer flora in Passamaquoddy Bay consisted of *Chaetoceros diadema, C. compressus, C. constrictus* and *C. socialis.*

In October in the Woods Hole region the main species were a mixture of temperate and boreal forms: *Rhizosolenia alata, Skeletonema costatum, Leptocylindrus danicus, Chaetoceros decipiens* and *Rhizosolenia setigera.* The winter flora of this region consisted mainly of boreal species: *Rhizosolenia hebata* v. *semispina, Thalassiosira nordenskioldii, Rhizosolenia fragillissima, Nitzschia seriata, Thalassiosira decipiens* and *T. nitzschioides* (109).

Southwestern Atlantic. In the southwest Atlantic and the Bellingshausen Sea, Hart (73) found that there are four main types of water which can be distinguished by the diatom flora they support. The eastern Weddell Sea is dominated by *Chaetoceros criophilum, Rhizosolenia styliformis* and *Corethron valdiviae.* Also present in considerable numbers are *Nitzschia seriata* and *Thalassiosira antarctica.* This flora is moderately rich in species and very rich in quantity. The western Weddell Sea is dominated by *Chaetoceros socialis, Thalassiosira antarctica* and *Chaetoceros neglectus. Fragilaria antarctica* and *Nitzschia seriata* are also very common. This flora is very rich in species with minute forms dominant.

The Bellingshausen Sea water is characterized by an abundance of *Corethron valdiviae, Thalassiothrix antarctica, Nitzschia seriata, Fragilaria antarctica, Chaetoceros neglectus* and *Rhizosolenia alata* f. *gracillima.* The water of Bransfield Strait, which is a branch of the Bellingshausen Sea, is dominated by *Corethron valdiviae.*

Southern Seas. Hendey (79), in his study of diatoms of the Southern Seas, recognized three floras: a warm water flora, a cold water flora and an antarctic convergence flora which is abundant in species and genera and is formed by the association of cold water and warm water. Owing to the cold Antarctic Drift which sweeps across the South Atlantic from Cape Horn past South Africa to the South Indian Ocean in almost circumpolar fashion, very few species from warm water ever reach the far south and become established. Although cold-water species sometimes associate with warm-water floras, very seldom if ever does a warm-water species become established in a flora which is subject to polar or subpolar conditions.

It is more important to consider a flora in relation to ocean currents which support them than in relation to actual degrees of latitude through which they spread. For example, the warm Brazil current running southward along the eastern seabord of South America enables a large warm-water flora to be supported to 50° S. On the other hand, in the eastern Atlantic and eastern Pacific Oceans cold upwellings of the waters of the Benguela and Peru currents enable these seas to support floras with distinctly colder facies. The disposition of land masses in the southern hemisphere and the currents which operate around them have marked effect on the geographical range of various species. Also the layering and zoning of surface water exerts a considerable effect on certain species.

In general three types of plankton, as defined by Lebour (107), are recognized: holoplankton, composed of true plankton forms; tychopelagic forms, bottom species which form chains that may break off and float but never reproduce in the plankton; meroplankton forms, dependent on the coast but sometimes found under oceanic conditions. However, all true oceanic species are holoplanktonic. The neritic flora may consist of holoplanktonic, meroplanktonic and tychopelagic species.

In warm-water floras the oceanic or holoplanktonic flora is not well developed. The solenoid forms are poorly represented and the discoid forms predominate. The genera *Chaetoceros* and *Bacteriastrum* are scarce, while the genera *Hemidiscus* and *Asterolampra* are very common. Several neritic species, such as *Fragilaria striatulam, Melosira sulcata* and *Planktoniella sol,* have been observed in the oceanic flora. The holoplanktonic species of the neritic flora are sometimes very characteristic of a given geographical area, such as the east coast of Africa which supports a flora that is very endemic. The meroplanktonic species seem to be common to all coasts enjoying a temperate climate.

The oceanic holoplanktonic flora of cold water is mainly characterized by such genera as *Corethron, Chaetoceros, Fragilariopsis* and *Nitzschia.* The normal habit of the discoid forms is solitary rather than colonial. In the oceanic plankton, besides the holoplanktonic forms we also find parasitic and adventitious species.

The parasitic diatom flora is that which inhabits the skin of whales and consists of true constituents, *e.g., Licmophora lyngbyei,* species of *Cocconeis* and *Climacosphenia,* and fortuitous ones, such as some neritic species. Hart (73) believes that after more study the diatoms in the skin film of whales may be used to indicate their movements, but now our knowledge of them simply indicates that the whale spends at least one month in the Antarctic.

The adventitious forms of the oceanic flora are those obtained from melting ice. The predominant species are truly neritic diatoms, and there is no evidence of any freshwater species from the mainland. The principal species are *Fragilaria curta* and *F. linearis.* Small numbers of *Navicula corymbosa* and *Amphiprora oestrupii,* which were mentioned by Grunow as being in the Arctic, are also present.

The neritic holoplanktonic forms of cold water are mostly solenoid species. Some discoids, *e.g., Coscinodiscus bouvet* which is a characteristic Antarctic species, are found. There are also present some species of *Coscinodiscus* which are well known in northern waters. The genera *Nitzschia* and *Chaetoceros* are abundant, but by far the most common genus is *Corethron.*

Antarctic. Mann (113), in his studies of diatoms from the Antarctic, found that the species were large elegant forms of which no small proportion seems to be of subtropical rather than of temperate or frigid origin. Indeed, many of the species were known previously only from the tropic or subtropic regions or as fossils from subtropic conditions. Many of them are known from Miocene deposits in various parts of the earth. He also noted the similarity of the Antarctic flora to that of the ornate floras of the Philippines and the East Indies.

Arctic. In contrast to that of the Antarctic, the Arctic flora consists of species which are simple in construction as to both shape and elaborateness of ornamentation, and they are very different from the tropical or subtropical species. Perhaps the reason for this is that the Arctic Ocean is practically cut off from tropical currents, while the Antarctic is easily accessible to the waters of warm currents, since the continents come to a point in the southern hemisphere.

Northeastern Pacific. In the northeastern part of the Pacific, Scotch Cape, Alaska, Cupp (47) found that the diatom flora consisted of pelagic species of arctic and temperate oceanic and neritic species. However, the temperate neritic species, which are characteristic of more northerly temperate regions, predominated. *Biddulphia aurita* reaches its maximum in April. It is followed by *Thalassiosira nordenskioldii* and *Chaetoceros socialis* in April and May, with *Chaetoceros debilis* becoming dominant in June or July. *Leptocylindrus danicus* reaches its greatest maximum in July. *Asterionella japonica,* a southerly temperate neritic species, predominates in the warmest months, September and October. The seasonal succession at Scotch Cape most closely resembles that in the Passamaquoddy region, New Brunswick, and the Gulf of Maine, except for the abundance of *Asterionella japonica* in September and October.

Eastern Pacific. In the eastern part of the Pacific the greatest amount of study has been in the waters adjacent to California. The flora here seems to be a mixture of northern and southern forms. The common occurrence in the subsurface plankton of such boreal-arctic oceanic forms as *Rhizosolenia semispina, Chaetoceros criophilum* and *Thalassiothrix longissima* is due to the upwelling of deep water which occurs in this region (171). Also strays from the north, *e.g., Asterionella kariana* and *Thalassiosira nordenskioldii,* occasionally appear in this region. *Thalassiosira* is the characteristic genus of the flora of Alaskan waters and serves to distinguish it from other floras (6). Typical southern species are *Eucampia zoodiacus* which is characteristic of the southern stations of this region and *Guinardia flaccida* which is sometimes encountered as a stray from the south (5).

Allen (13) noted in his study of marine diatoms in the waters off the coast of southern California that the setose genera, *Chaetoceros,* for example, were dominant in the spring, and in the summer the filiform genera, *e.g., Rhizosolenia, Thalassionema* and *Thalassiothrix,* were common.

The flora of the Gulf of California may be divided more or less into three parts: the southern section of the outer gulf, which is an oceanic flora; the middle section, which is a neritic flora characterized by *Coscinodiscus wailsii;* and a northern section in which *Asterionella japonica, Chaetoceros radians, C. compressus, C. debilis* and *Skeletonema costatum* are common forms.

In the Gulf of Panama Allen (11) found some striking similarities both quantitatively and qualitatively with the diatoms of northern waters. The abundance of *Chaetoceros debilis* and the common occurrence of *Nitzschia seriata* were notable. These two species have been found to be of common occurrence in northern Pacific waters. The abundance of a planktonic flora of diatoms which is often associated with waters of northern latitudes was noted here. Though this region differs in many ecological factors from northern seas, it is similar in one major way, that is, in the inundation of the coast line. The coast line of the Gulf of Panama is much more inundated than that of most tropical seas. Thus more nutrients are provided by run off. Another interesting parallelism is the occurrence of certain species and endemic varieties in the Philippine Islands and Campèche Bay in the Gulf of Mexico. It is difficult to explain how they arrived at their present distribution, for they are not found in intervening regions (112).

Western Pacific. As for the western Pacific, Aikawa (1) came to the conclusion that the various sea areas about Japan might be divided into neritic and oceanic groups according to their plankton. He recognizes five oceanic communities: the Kurosio group, the Tusima current group, the Oyasio group, the Liman group and the Yellow Sea group.

The Kurosio group may consist of desmoplankton, styliplankton, triposplankton, or discoplankton. One or two of the following species are generally always present: *Climacodium biconcavum, C. frauenfeldianum, Dactyliosolen tenuis, Gossleriella tropica, Guinardia flaccida, Hemiaulus heibergii, H. hauckii, Planktoniella sol, Streptotheca indica, Ditylium sol.* There are also several species of *Rhizosolenia.* The relative abundance of *Rhizosolenia* usually corresponds to the purity of the Kurosio group.

The Kurosio group is found in the tropical regions around the Marianne and Western Caroline Islands, in the seas off the Philippines and off Formosa as far as around the Bonin chain. Aikawa applies the name, Tusima current group, to the Kurosio group which is found along the west coast of Kyûayû in the Japan Sea after passing through the Tusima Straits. Though modified several times by neritic groups, the flora of this current always retains its Kurosio character. In the southern Japan Sea the Tusima current covers a wide expanse a little distance off shore. North of the Noto Peninsula or of Sadoga Lima it is usually restricted to a sisted of pelagic species of arctic and temperate oceanic and neritic species. However, the temperate neritic species, which are characteristic of more northerly temperate regions, predominated. *Biddulphia aurita* reaches its maximum in April. It is followed by *Thalassiosira nordenskioldii* and *Chaetoceros socialis* in April and May, with *Chaetoceros debilis* becoming dominant in June or July. *Leptocylindrus danicus* reaches its greatest maximum in July. *Asterionella japonica,* a southerly temperate neritic species, predominates in the warmest months, September and October. The seasonal succession at Scotch Cape most closely resembles that in the Passamaquoddy region, New Brunswick, and the Gulf of Maine, except for the abundance of *Asterionella japonica* in September and October.

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The Oyasio group contains *Corethron hystrix, Thalassiothrix longissima, Thalassiosira nordenskioldii, Thallassiosira decipiens, Denticula* sp., *Coscinodiscus* sp. and *Biddulphia aurita.* In summer some southern forms are intermixed. The group is found in summer in the Okhotsk Sea and around the western Aleutian Islands. In December it was off Hokkaido, and in January it was around Rasyôwa Tô.

The Liman group is located in the northern part of the Japan Sea and has a marked influence over the area near the Tugaru Straits. In winter it is along the east coast of Tyosen as far as Uturyô Tô, while in summer it recedes to the area off Vladivostok. It is characterized by *Corethron hystrix, Thalassiosira longissima* and *Coscinodiscus* sp. In summer such southern forms as *Rhizosolenia hebetata* are also present.

The Yellow Sea group is greatly influenced by the Kurosio group. The flora is poor as to species and consists of neritic species together with some southern forms.

In the neritic communities the geographical influences play an important rôle in determining the numbers and kinds of diatoms present. The diatoms are rich in both numbers and species. The genus *Chaetoceros* is always a leading group. These facts are generally true regardless of the influence of either the Kurosio or Oyasio group. Simple floras consisting of *Chaetoceros, Thalassiothrix, Nitzschia seriata, Skeletonema costata* and other neritic forms often make up the neritic floras on the northern Pacific Coast and on the shores of the Sea of Japan, but seldom occur in the southern Pacific. Seasonal succession is very marked in the neritic communities of the Japan Sea and the Tohoku region where plankton groups of different origin influence the neritic community throughout the year. However, the neritic communities of the southwest Pacific, which are influenced only by the Kurosio group, remain remarkably stable throughout the year.

Maxima. As a rule, oceanic communities are not so rich as neritic ones. Thus we may have a thin population which is often uniform in composition, spreading out over a wide area in oceanic communities, while the neritic ones are much more limited in extent. The diatom production in cold waters was very abundant quantitatively, while that of warm water was richer in species number.

It is interesting to note the similarities and differences in times of diatom maxima throughout the world. There is great similarity in the times of diatom maxima in the waters of the eastern and western North Atlantic. In both cases there is a distinct spring maximum and a smaller fall maximum. In the southwest Pacific at Great Barrier reef the amount of microplankton is remarkably stable except for an increase in August (114). In the coastal waters of New South Wales the spring maximum begins in August. The autumn maximum does not start till January and reaches its peak in February. In Aomori Bay, Japan, the richest diatom flora occurred on September 1, 1929, and from October 16 to November 1, 1930 (92). At Scotch Cape Light the diatom maxima were similar to those of temperate and northerly regions, that is, there were a strong spring and a lesser fall maximum. At Friday Harbor, Washington, there was an almost continuous maximum from May till September which is similar to conditions at Karajakfjord Grunland (135). In southern California waters the greatest maximum occurs in the first third of the year (47). However, as brought out in the consideration of the ecological factors effecting distribution, it is the type of nutrient supply which largely determines the kind and extent of diatom dominance.

Also it must be remembered that no latitude should be considered the most productive for diatom growth, for though northern latitudes are generally considered more productive, very large catches have been reported from the Gulf of Panama and along the coast of Central America. Likewise the diatom population at La Jolla and Point Hueneme in southern California waters and Friday Harbor in Washington have been found to be larger than those in Scotch Cape, Alaska.

Summary. From the above review of the literature it is evident that both geographical and ecological factors together, or either of them, may serve as isolating mechanisms in the distribution of marine diatoms. It is the combination of these factors which enables geographical regions and ocean currents to have at a given time characteristic floras. It is not only the kind of species but their relative abundance which gives a flora its individuality. Through the study of these floras much has been learned about ocean currents. As a rule, the neritic species are more endemic to certain geographical areas than the oceanic species. However, only a very few oceanic species have been found to be bipolar. Further study of the factors determining diatom distribution and abundance will teach us much concerning the productivity of the seas.

DIATOMS OTHER THAN MARINE SPECIES

Ecological Factors

General. Before considering the more specific geographical and ecological conditions which affect fresh-water diatom growth, it might be well to consider the more general habitats afforded fresh and brackish-water diatoms and how they are adapted for such habitats. The plankton diatoms are those which live in the surface waters and spend all or most of their life afloat. They are either structurally or physiologically adapted for this manner of life, as has already been described for marine plankton forms. Plankton diatoms vary a great deal in size and might roughly be divided into small forms or nanoplankton, and large forms or net plankton. The nanoplankton consists, among others, of *Stephanodiscus pusillus, Cyclotella dubia, C. meneghiniana, C. kutzingiana* and *C. comta.* The net plankton owe their size to colony formation or to the size of the individual. To this group belong *Rhizosolenia, Synedra, Asterionella, Melosira, Fragilaria, etc.* Though usually plankton diatoms are in some way particularly adapted for this mode of life, there are some genera, *e.g., Nitzschia, Surirella* and *Cymatopleura,* which are found in plankton or littoral condition.

Bottom Forms. The bottom diatoms are those which live on the substrata. Most of them possess raphes and are able to move about. They may live in shallow or deep water, depending on the light penetration and the amount of O_2, H_2S, CH_4 and CO_2 present. Temperature also limits their distribution. In shallow water in very cold weather the benthic flora is greatly reduced. This flora is often well developed in lakes and ponds, and in streams and rivers in places where the current is not too swift. To this flora belong *Navicula, Surirella, Nitzschia, Pleurosigma* and *Campylodiscus.*

Epiphytic Forms. The epiphytic forms are those which attach themselves by a secretion of jelly to the substratum. This jelly may form a cushion, in which the diatoms live, or stalk-like structures, as are found in *Cymbella* and *Gomphonema.* In other cases by the secretion of jelly the whole valve may be attached to the substratum, as in the genera *Achnanthes* and *Cocconeis.* Diatoms may live epiphytically on a great many different types of substrata. The commonest are rocks and rooted vegetation. However, Kolbe (94) reports that *Gomphonema olivaceum* has been found abundant on the fungus *Sphaerotilus.* He also states that *Nitzschia* has been noted living endophytically in *Sphagnum* leaves. It is also true that certain species of *Vanheurckia* and *Eunotia* often live in the tops of sphagnum plants. I do not believe, however, that it has been demonstrated that they are epiphytic. Because of their method of attachment it is only this type of diatom which can live successfully in places where a swift current is present. They are often found on the surfaces of rocks over which the water flows, and are often referred to as "rheophiles" or "rheobronts". Hustedt found in his studies of the diatoms of the East Indies that of the endemic species over half were rheophiles.

Water-Type Forms. In considering the distribution of fresh-water diatoms, the type of water mass, whether it be lake, pond, moor, river, stream or spring, is important in determining the type of diatom flora in it.

A lake is differentiated from a pond mainly by conditions associated with its depth. In both, the diatom flora consists of benthic, epiphytic and planktonic species. The degree of development of these various types depends on the physical conditions present. If a broad littoral zone is present, the lower part of which is occupied by rooted vegetation, then a well developed epiphytic and benthic flora may be present. The plankton species find their best development where there are large expanses of open water. Particularly in fairly deep waters which have spring and fall overturns do we find great masses of plankton diatoms which are often referred to as spring and fall blooms. Just what factors are involved in initiating a bloom seem to vary depending on the lake. In Lake Michigan (52) and Lake Erie (32) turbidity of the water seems to greatly influence the amount and duration of diatom bloom. In Lake Erie (32) wind, precipitation and solar and sky radiation are also very important. Nitrogen and phosphorus never seem to act as limiting factors (129, 130, 177). In contrast to these findings is the work on English lakes which seems to emphasize the importance of dissolved salts in relation to diatom productivity. Pearsall (129) states that most diatoms must have a calcium carbonate hardness above 3, and silica above 0.5 mg. per liter, in order to occur in abundance. Thus it would seem than any one of several factors may limit diatom production.

As a rule, the spring diatom bloom is much larger than the fall bloom, though some exceptions may occur (133, 30, 31, 62, 28, 49, 53). Though some diatoms may exhibit both a spring and fall pulse usually the dominant species are different. As noted by Chandler (30), those genera which are usually dominant in the spring bloom—*Asterionella, Synedra, Fragilaria*—belong to the Pennales, while those which are dominant in the fall pulse—*Melosira, Cyclotella, Stephanodiscus*—belong to the Centrales. Of course, all genera may occur at any time, but their time of dominance varies. For instance, many workers have noted that *Asterionella* usually occurs earliest in the spring, and is then succeeded by other species. Pearsall (130) believes this succession is due to the fact that *Asterionella* has a higher nutritive requirement than the succeeding species. Akehurst (2), however, believes that this succession is due to the fact that one group produces substances which are toxic to itself but stimulating to another group. For example, *Asterionella* would produce a substance toxic to itself but which would stimulate *Synedra*. Thus a *Synedra* pulse would follow an *Asterionella* one.

The kinds of species detected in these various habitats are dependent upon whether the water is eutrophic, oligotrophic or dystrophic (173, 122). The eutrophic water, rich in dissolved nutrients, is often characterized by *Stephanodiscus binderanus, Cyclotella dubia* and *Asterionella formosa,* also by various species of the genera *Cymbella* and *Gomphonema* (105). Oligotrophic water, low in dissolved nutrients, is typical of mountainous streams and glacier lakes, but may be found under any condition which produces a low level of dissolved nutrients. The plankton is usually poorly developed (148). *Cyclotella comta, C. kutzingiana, Stephanodiscus astraea* and *Tabellaria fenstrata* may be gathered under these conditions.

A similar classification of lakes is that of Pearsall (128) in which he divides them into rocky and silted lakes. The rocky lakes, corresponding to the oligotrophic group, are poorer in Ca, Si, CO_3

and organic water. The diatom flora is not so well developed as that of other algae. In one such lake *Surirella robusta* v. *splendida* was a constant species. Silted lakes are richer in Ca, Si, CO_3 and organic matter, and thus correspond to the eutrophic group. They have a richer diatom flora.

Sometimes it so happens that oligotrophic conditions develop in a eutrophic lake. This is particularly true after the diatom bloom of a species with fairly high nutritive requirements. Thus *Melosira granulata* is often considered eutrophic because it occurs in eutrophic lakes. Actually it is oligotrophic because it occurs after a *Synedra* bloom (87).

Dystrophic water is that which is high in humic acid and low in dissolved nutrients and oxygen. The water of bogs and swamps is usually of this type. In the beginning of the formation of a bog the water is often eutrophic, being rich in dissolved nutrients and circumneutral in reaction. As it progresses in formation the oxygen and dissolved nutrients become poorer and the humates richer. A low pH develops. A rather specialized flora can live under these conditions. Some of the species have been found to be aerophile and live in the tops of sphagnum and other moss. Under such conditions various species of the genus *Eunotia* together with certain species of *Pinnularia, Vanheurckia* and *Stenopterobia* are found (125, 106, 94).

In contrast to these more or less quiescent types of water are those which are flowing. The amount of current greatly influences the kinds of diatoms which may be present. Allen (4) points out that water current above a very modest speed is distinctly inimical to plankton development. Likewise, slime forms are not able to develop where the current is rapid. Indeed, in fast flowing streams only those forms which can attach themselves by gelatinous mass or stalks can survive. Thus the typical genera of such habitats are *Achnanthes, Cocconeis, Cymbella* and *Gomphonema*. *Ceratoneis arcus* is also considered a typical stream species (61).

The amount of current has also been found to affect the shapes of diatoms. *Desmogonium* in fast flowing water is long with scarcely capitate ends, while in standing water it is short with broad capitate ends (87).

Plankton development is usually scarce except in places where the current is reduced. Often along the edges of streams or in little pools where the current is not very great, a benthic flora will develop.

Not only does the current affect the availability of diatom habitats but also the amount of dissolved nutrients, temperature, oxygen and turbidity of the water. Thus in muddy rivers a very poor diatom flora is present, whereas in a clear river or stream excellent conditions may develop for diatom growth. A mountainous stream with high oxygen, and low temperature and mineral content will support a very different diatom flora than that of the open plain where the current and dissolved oxygen are often less and the temperature and dissolved mineral content higher (106, 25, 94). Due also to erosion and spotty pollution a great variation may exist as to the dissolved nutrients and oxygen available for growth.

Rivers are not unlike streams in that they are characterized by having masses of constantly moving water which is thoroughly mixed and not stratified as in lakes. The rate of movement of the water greatly influences the temperature, dissolved nutrients and gases. Rivers are usually larger and have regions of slower water movement so that plankton often develops better than in streams. Indeed, the Volga, the Thames, the Illinois, and the San Joaquin have been shown to have well developed plankton floras (136, 91, 4, 18). The main genera are *Asterionella, Cyclotella, Diatoma, Melosira, Nitzschia, Synedra* and *Stephanodiscus*. Just when these genera reach their maxima seems to be more closely correlated with temperature of the water and the dissolved nutrients and gases than with the calendar month of the year. Allen (4) found that *Bacillaria paradoxa* was the foremost plankton species during the flood season. The source of the species which make up the plankton of a river seems to vary. In some cases the species seem to be derived from the head waters (105, 91), while in other cases they appear to come from diatom associations in the more shallow parts of the river (136). A special type of plankton formation, which has been called a "neuston" (94), is often found near where waste water enters a stream or river. The diatoms form a thin film over the surface of the water. Schroeder (148) found in the Mulde River that *Nitzschia palea* was the main inhabitant of this film, but concluded that almost any slime diatom which is not too heavy might live in the neuston.

Besides the plankton flora of a river, a well developed benthic and epiphytic flora may exist if littoral conditions are suitable. Along the edges of the river in the so-called spray zone there may be many aerial forms which are often the same species as those in similar habitats on the edges of lakes and ponds.

The typically rheophilic diatoms sometimes contain the most characteristic or endemic species of a region. Hustedt found in his studies of the diatoms of the Dutch East Indies, that of the 222 endemic species or varieties, 131 were rheophiles (87).

Another type of flowering water habitat is that of springs. The pool spring is a much better diatom habitat than the spring which forms a waterfall. The cool well aerated water makes a suitable habitat for many species usually found in streams and rivers. Especially suited to such habitats is *Odontidium hiemale* v. *mesodon* (94). The flora of hot springs, contrary to what might be expected, consists of eurythermal species of the flora of the region (133, 54, 131, 94, 98, 87). In the Dutch East Indies the flora of thermal springs was found to be the same as that in running water of 45° C.

Aerial Forms. A third general habitat of fresh-water diatoms is that in which the diatoms are more or less exposed to the air.

Such aerial habitats are in moss, on trunks of trees, on damp stones and leaves, and in the soil. Species which can live in such habitats must be able to endure much more rigorous environmental changes than those which live in water. They must be able to withstand flooding as well as extreme drought, and also sudden changes in temperature. Thus, as would be expected, the diatoms which can survive such changes represent a small and specialized group. For the most part they are small forms. Very few large diatoms are found under dry conditions. Several different classifications of aerial habitats for diatoms have been proposed (16, 94, 132, 133). Perhaps the most convenient division is into those which live in moist aerial conditions, and those which are subject to truly dry conditions.

Under the group of moist aerial habitats are included wet rocks and moss, caves, snow and ice, and the spray or surf zones of lakes, rivers and oceans. In such habitats are located typically aerophile forms which can live in water or out of it.

On rocks or moss kept wet by seeping springs are often located *Pinnularia borealis, Melosira roseana, Navicula fragilarioides* and *N. confervacea;* also certain species of *Cymbella, Gomphonema, Synedra, Achnanthes* and *Cystopleura.* In very wet moss, such as the tops of *Sphagnum,* species of the genera *Eunotia* and *Vanheurckia* are abundant.

Cave and Spray Forms. Caves also furnish a suitable habitat for diatoms if light is not too limiting a factor. The very moist atmosphere permits a great variety of diatoms to flourish, including *Fragilaria construens* v. *venter, Melosira dickiei, M. roseana, Navicula kotschyi, N. perpusilla* and *Pinnularia borealis* (147, 84, 94).

Another interesting habitat for diatoms is the spray zone of lakes, rivers, *etc.* Though the species in this zone are often found in other habitats, they all belong to genera which produce a jelly sheath. Close to the water's edge are *Achnanthes, Cymbella, Gomphonema, Cystopleura* and *Denticula.* Hustedt found the genus *Gomphonema* sometimes high above the water's edge where only spray produced by the strongest wave action could wet it. This genus seems to be better able to resist drying out than *Cymbella.* Perhaps this is due to the greater amount of jelly in the tree or branched colonies than in the linear ones.

Dry-Habitat Forms. The diatoms which live in a truly dry atmosphere are the soil diatoms, those that live on only slightly damp rocks or moss, and those that live on leaves. Our knowledge of diatoms which live in the soil is still quite limited, and no doubt many new species will be found in this specialized habitat. For the most part they are small, belonging to the Pennales. It is believed that because they have a raphe and are able to move to deeper regions when the soil dries out, they are able to live in this more or less unfavorable habitat. Diatoms are less often encountered in woods soil than in field or garden soil, and are most plentiful in the top 10 cm. *Hantzschia amphioxys, Navicula atomus, N. nitrophila, N. mutica, N. contenta* f. *biceps, Pinnularia balfouriana, P. brebissonii* and *P. borealis* (24, 131, 78, 119, 56) are the commonest soil species.

As for diatoms which can live on dry rocks, Krasske (98) has reported *Eunotia fallax* v. *gracillima, Melosira dickiei, Navicula contenta, N. krasskei* and *N. sohrensis* living among *Protococcus.* The species which can live in such habitats are rather limited in number.

The extent to which diatoms will occur on dry moss seems to be limited for the most part by the drying of the moss, the amount of light and the temperature of the substrata. Cool, damp, humus-rich moss of kaolinic rock will have more species than warm, dry, humus-poor moss on limestone rocks (16, 94). Diatoms which can live in such habitats must be able to withstand extreme variation in moisture conditions and thus great variation in salt concentration. Usually the smaller varieties rather than the typical species are found in dry moss. For example, *Navicula contenta* v. *parallela* and v. *elliptica* instead of *N. contenta; N. mutica* v. *cohnii* instead of *N. mutica;* smaller forms of *N. fragilarioides* and *Pinnularia borealis;* also the smaller forms of *Melosira roseana* rather than the long filamentous varieties of *spiralis* and *epidendron* (101). Some aerophiles have been found to store a large amount of oil. Often diatoms living in such habitats build inner plates, as found most commonly in *Melosira dickiei* and *M. roseana.* Kolbe (94) believes this adaptation to be more closely correlated with drying out, while Husted (86) associates it with increased salt concentration. The reduction in size, increase in oil storage, and the building of inner plates may well be special adaptations of these species to withstand drying out and increased salt concentration (94, 86). The diatoms occurring in such habitats are for the most part geographically widely distributed species, though a few endemics have been found.

Other Forms. Whether or not moist leaves furnish a separate habitat for diatoms is still uncertain. Indeed various species have been reported from moist leaves in tropical forests (176, 175, 86) or on leaves sprayed by mist from a water fall. The species were mostly stream or lake forms (94, 86). The one truly atmospheric species was *Navicula contenta* v. *biceps.*

Summary. Thus we see that there are many physiographic conditions which may furnish suitable habitats for diatoms. Though a single species may occur in several of these habitats, the composition of the flora of each varies and is characteristic of each one. The reason why a given habitat supports a certain flora is that it provides a certain balance between ecological factors which make possible that particular type of diatom growth. As has been previously said, any one of these factors may become limiting and thus change the type or prevent diatom growth.

It is well perhaps to examine these factors and find out just what is known as to how they affect diatom growth so that we may better understand their action in combination with other factors. As yet little is known concerning the nutrition and growth of diatoms. No doubt further research may reveal more important factors than those now known. However, from present studies it would seem that it is the chemical content of the water together with light and temperature which determine the species to be found. Some elements are utilized in relatively large amounts and others in small amounts. The following chemical elements, radicals and compounds have been found to be important in diatom nutrition and growth: sodium, calcium, iron, silicon, nitrates, phosphates, sulphates, oxygen, carbon dioxide and humic acid.

Salinity. Sodium chloride was the first salt that was recognized as important in determining diatom distribution, and water was classified as marine, brackish or fresh. With more investigation a more refined system was needed and several were suggested. The most satisfactory was that of Kolbe (93).

He divides diatoms into four groups—polyhalobiens, euhalobiens, mesohalobiens, oligohalobiens. The polyhalobiens are species which can stand a salt concentration greater than that of the sea. To this group belongs *Navicula longirostris.* The euhalobien species develop best in a total salt concentration of 30–40% (NaCl 17–20%). To this group belong the marine and brine water species. The mesohalobien species have their optimum in a total salt concentration of 5–20% (NaCl 2–15%). To this group belong such brackish water species as *Achnanthes brevipes* v. *intermedia, Amphora coffeaeformis, Nitzschia hungarica, Stauroneis salina* v. *latior, Navicula salinarum, N. integra, N. pygmaea* and *Diploneis interrupta.* The oligohalobien species have their optimum condition in water with a very low salt concentration. This group is subdivided into those species which have their best development in water with a small amount of salt, such as *Navicula cincta, Anomoeoneis sphaerophora, Caloneis amphisbaena, Cyclotella meneghiniana, Diatoma elongatum* and *Navicula hungarica.* These are known as halophil species. Those fresh water species, such as *Diploneis elliptica, Cymbella lacustris, Gyrosigma attenuatum, Melosira arenaria* and *Hantzschia elongata* which are not sensitive to a little salt, are known as indifferent species, whereas those which live in very pure water and dislike salt are halophobes. To this group belong most species of *Eunotia, Tibiella, Stenopterobia* and *Tabellaria flocculosa,* and some of *Pinnularia* and *Vanheurckia.* These species are often referred to as sphagnophils, because the waters containing them are usually rich in humic material. It is hard to be sure whether it is the lack of salt or the accumulation of humic materials that they like.

Sodium chloride is certainly one of the most important salts which limit the distribution of diatom species. Indeed diatoms may be generally classified into those which are specific for certain salt conditions and those which are euryhaline or indifferent. Richter (137) has noted that with *Nitzschia putrida* it is the sodium which is the important atom of the compound in diatom nutrition, and which causes the influence of the salt on diatom development. In other diatoms it would seem that it is the chlorine which is the important atom. Petersen (133) found in his study of the lakes and bogs of Denmark that the threshold of the effect of the chloride factor was at about 100 mg. Cl per liter. Where the chlorine content was below this amount indifferent species formed 80–95% of the flora. Above 100 mg. Cl per liter these species dropped off to 56–70% of the flora and halophilous and mesohalobous species increased correspondingly. If the chlorine content was between 16–42 mg. per liter it was of no significance, since other factors were more important. These findings seem to contradict the opinion of Kolbe (93) and Budde (26, 27) that the chlorine content must be much higher in order for water to be classified as brackish.

The so-called brackish water habitats are in the main of three types: those which are formed by the mixing of fresh and salt water; inland salt lakes; and waste water of various industries. The first type formed by the mixing of fresh and salt water contains mainly euhalobien and oligohalobien species. The littoral flora, frequently very rich, is composed of many characteristic species which are often fairly restricted geographically. The inland salt lakes, if old, contain many mesohalobiens. If new, there are fewer species, but they are rich in number. The brine or waste waters contain very few species. However, if the salt content is less, the flora may be richer. A salt content of 10% (98) was found to support *Navicula longirostris, Nitzschia epithemoides* and *Navicula cincta* v. *heufleri.* A salt content of 9% (85) supported *Amphora coffeaeformis, Navicula longirostris, Nitzschia epithemoides* and *Nitzschia frustulum.* In a salt content of 3% *Navicula gregaria* was abundant.

Calcium. Calcium seems to be one of the more important elements in diatom nutrition, but just what is its most important rôle is not known. It is a part of the buffering system which controls pH. It also acts as an antagonizer and counteracts the toxic effect of excesses of various ions. It controls the amount of H_2SO_4 in the water, which is produced by bacteria and might otherwise be toxic. The amount of calcium in solution is somewhat dependent on whether oligotrophic or eutrophic conditions exist. The soluble calcium bicarbonate is often changed to the insoluble calcium carbonate in the epilimnion. As such it settles to the bottom and out of solution in oligotrophic lakes. However, in eutrophic lakes the bacterial activity changes it back to the soluble bicarbonate form, and thus a higher calcium content is maintained (94). That many diatoms definitely like calcium-rich waters is evidenced by the fact that diatom phytoplankton predominates when the $\frac{Na+K}{Ca+Mg} < 1.5$. However, it may be due to the effect which calcium has on the availability of other elements. When the $\frac{Na+K}{Ca+Mg}$ ratio is low, water is usually rich in nitrates, carbonates and silicates (126). Pearsall (128) goes even further and states that in calcium-poor waters carbohydrate-producing organisms dominate, while in calcium-rich water fat-producing species dominate.

Some species of diatoms seem definitely to prefer calcium. All species of *Synedra* seem to like some calcium, but it seems especially important for the development of *Synedra acus* and its varieties. *Microneis minutissima, Gomphonema olivaceum* and certain species of *Cymbella, Diploneis* and *Navicula* have been found living in close association with deposits of calcium carbonate (178, 94). The calcium-iron ratio seems to be important to some diatoms. *Tabellaria flocculosa* seems to demand a very definite calcium-iron ratio. Other species seem to dislike calcium and are referred to as "calciophobes". They are many species of the genera *Eunotia, Stenopterobia* and *Tibiella* and certain species of *Pinnularia, Vanheurckia* and *Fragilaria.*

The effects of calcium seem to be very closely linked to those of pH. Indeed in many inland lakes the buffering system is that of calcium bicarbonate and carbonic acid. This is particularly true of oligotrophic and eutrophic lakes. In dystrophic lakes the pH is largely controlled by the amount of humic acid present. In the epilimnion, where the phytoplankton activity is great and the carbon dioxide is used up, the alkalinity is higher than in the hypolimnion where the carbon dioxide and carbonic acid accumulate.

The species which make up a flora seem to be greatly influenced by the pH of the water, and waters of different pH have very different floras. Very few species can live in a water with a pH below 3.5; though the flora may be rich quantitatively it is poor qualitatively. Species found in such acid waters are *Tabellaria flocculosa, Eunotia trinacria, E. exigua* and *Navicula subtilissima* (148). From a pH of 3.5 to 6 are many species of *Eunotia* and some of *Vanheurckia, Stenopterobia* and *Pinnularia* (148, 87, 125). In the circumneutral range is the greatest diversity of species (94, 125). The more alkaline waters, those with a pH above 8, also often show a more or less restricted flora. Patrick (125) found in her Pocono studies that two lakes with the same pH but different calcium contents might support very different floras.

In considering the effect of pH one should think not only of its direct effect upon the organisms, but, what is even more important, of its indirect influence on the solubility of various substances. Also very acid lakes often support much less life than circumneutral ones, and thus the supply of oxygen and carbon dioxide may be much more limited.

Iron. Also iron is much more soluble in acid than in circumneutral waters. Uspenski (174) believed it to be as selective a factor in diatom growth as NaCl. Typical inhabitants of iron-rich water are many species of *Eunotia,* some large forms of *Pinnularia, Stauroneis phoenicenteron, Anomoeoneis serians* v. *brachysira, Pinnularia subcapitata* v. *hilseana, Stenopterobia intermedia, Surirella delicatissima, Surirella linearis, Gomphonema acuminatum, Pinnularia microstauron* (148, 94).

Silicon. Though silicon was long believed to be a necessary element in diatom nutrition it was not until 1904 that it was experimentally proven to be so (137). There seems to be a correlation between the calcium and silica content of the water. The more alkaline waters often have a high silica content, and the dead shells of diatoms often are found to be badly corroded (94). It has been pointed out that there is a decrease in dissolved silica in the water following a diatom bloom (116).

Nitrogen. The nitrogen-phosphorus ratio seems to be as important in the growth of fresh-water diatoms as in that of marine forms. In oligotrophic water the nitrogen is often so low that it is hard to detect by analytical methods, while in eutrophic waters, which are relatively high in nitrogen, it may be only 1–2 p.p.m. (94). Much more research is needed to understand correctly the qualitative and quantitative relationships of diatom growth to the phosphate-nitrate ratio. Some diatoms, such as *Diatoma vulgare, Navicula viridula, Nitzschia linearis* and *Surirella ovata,* seem to favor a high percentage of nitrates, whereas other diatoms, such as *Melosira granulata,* though growing in a eutrophic lake, actually prefer a low nitrate-phosphate ratio, for they occur after the nutrients have been exhausted by other diatoms (87).

Nitrogen may occur in water as nitrites, ammonium or nitrates. It is the nitrate form which is most utilized by diatoms. Diatoms may also sometimes utilize organic nitrogen and phosphorus (137, 138).

Sulphur. Sulphur is found most commonly as hydrogen sulfide or in the sulphate form. The hydrogen sulfide, which is largely due to bacterial activity, is often in relatively high concentration in the ground slime or in stagnant water. *Cyclotella meneghiniana, Caloneis amphisbaena* and many species of *Nitzschia, Campylodiscus* and *Surirella* are often found under such conditions (94). In habitats where the hydrogen sulfide was 3.9 parts per million, *Achnanthes affinis, Cymbella ventricosa, Hantzschia amphioxys* and *Nitzschia palea* were abundant. In hydrogen sulfide concentration of 1.5–3.7 parts per million, *Cyclotella meneghiniana, Neidium bisulcatum, Navicula minima, Nitzschia ignorata, Nitzschia tryblionella* v. *debilis* and *Surirella ovata* v. *salina* occurred.

In the Mulde River sulphate seemed to have an effect similar to that of chlorine on the diatom flora. In a concentration up to 200 parts per million no effect on the diatom flora was noted. In waste water with a sulphate concentration of 6,000 parts per million and a chlorine content of 45 parts per million, mesohalobe and halophile species such as *Caloneis amphisbaena, Navicula halophila, N. peregrina, N. radiosa* and *Nitzschia frustulum* v. *subsalina* were found.

Copper. Copper, which is usually poisonous to many algae, may be tolerated by diatoms in small amounts. In a concentration of 1.5 milligrams per liter, *Fragilaria virescens, Synedra ulna, Achnanthes affinis, Neidium bisculcatum, Navicula viridula, Cymbella naviculiformis, Cymbella ventricosa, Gomphonema parvulum* and *Nitzschia palea* were found living. In a concentration of 2.1 milligrams of copper per liter, only *Achnanthes affinis, Cymbella ventricosa* and *Nitzschia palea* were able to live (148).

Oxygen. Though oxygen is an important element for diatom growth, and the supply of it may act as a limiting factor, much is yet to be learned as to the classification of diatoms according to oxygen requirements. It is true that the three generally recognized types of water—oligotrophic, eutrophic, dystrophic—have very different oxygen contents and support characteristic diatom floras. Perhaps it is the difference in oxygen content that is one of the factors which limit the type of diatoms found. Most species of diatoms seem to be eu-oxybronts. In the Arctic region where the water usually has a high oxygen content, diatoms are abundant (127). However, many species of *Surirella* and *Campylodiscus, Cymbella ventricosa, Hantzschia amphioxys* and *Nitzschia palea* seem to be meso-oxybronts, since they typically occur in ground slime where the oxygen content is limited (87, 94). Of course, diatoms are continually adding to the oxygen supply in the waters in which they live, and their rôle in purifying water should not be overlooked.

Carbon Dioxide. Carbon dioxide, like oxygen, is a substance which undoubtedly is important for diatom growth, but as yet little is known about the specific requirements of diatoms for it. In most waters the animals and bacteria present furnish a sufficient supply. Eddy notes that *Synedra* seems to flourish on addition of carbon dioxide. Likewise, in some culture experiments which I have run with *Nitzschia linearis* and *Surirella biseriata,* addition of carbon dioxide seemed under some conditions to improve their growth. Ketchum also found in the culture of *Nitzschia closterium* that addition of carbon dioxide was beneficial in the mass culturing of this species.

Organic Nutrition. The rôle of organic substances in diatom nutrition, though recognized, is not clearly understood. It is true that dystrophic waters, which have a high humic acid content, support a very characteristic diatom flora. However, the fact that such waters are usually low in certain inorganic salts commonly found in other waters may be the favorable factor (94). Petersen (133) found that certain lakes and bogs which had similar chlorine, hardness and pH range, supported very different diatom floras. The most obvious difference was in the amounts of humous substances present. In culturing experiments it has been shown that the growth of diatoms may be benefited by addition of small amounts of soil extracts or natural water. It may be the organic matter or it may be the small amounts of certain minerals so added that is beneficial (94).

In nature high organic content of water is often associated with poor aeration, so it is hard to separate the two factors. The species which seem to be favored by high organic content are *Anomoeoneis exilis, Cocconeis placentula, Eunotia veneris, E. lunaris, Fragilaria crotonensis, Gomphonema olivaceum, Navicula radiosa, Pinnularia mesolepta, Stauroneis anceps, Stauroneis phoenicenteron, Tabellaria fenestrata, T. flocculosa* and *Vanheurckia rhomboides* (61, 136). However, other species, such as *Amphora ovalis* and *Gyrosigma attenuatum*, seem to be adversely affected by organic matter (136).

The value of such organic substances as thiamin, though suggested by Hutchinson (89), has yet to be proved.

Light. Besides the above chemical factors there are certain physical factors which seem to be very important in determining diatom growth. Just as in higher plants there are some species which prefer abundant light, while others live in regions of low light intensity. Those diatoms which prefer abundant light are usually found in the plankton or in the shallow littoral zones (148). Sunshine seems to favor the development of *Cyclotella meneghiniana, Fragilaria capucina* and *Navicula cryptocephala* (136). Diatoms which seem to tolerate low light conditions are those which live in caves, such as *Melosira roseana,* and those which can live in ground slime in deep water, such as species of *Campylodiscus* and *Surirella,* and *Synedra acus* (97). The fact that in winter diatoms seem to grow in shallower water than in summer may be due to the less amount of light penetration (61).

Temperature. The effect of temperature on diatom growth seems to be both direct and indirect. The direct effect is seen in the fact that certain diatoms are stenotherms and are found only in cold or warm water. Such species are characteristic of our Arctic or Tropic regions. Other species seem to be eu-eurytherms and are tolerant of a wide temperature range. Also diatoms vary as to the season of the year when they are most abundant, thus perhaps reflecting the effect of change of temperature. The indirect effects of temperature are equally if not more important, for the temperature of the water affects the solubility of the salts and also the bacterial activity. It is also temperature which determines the spring and fall overturn in a lake and thus the supply of nutrients. The melting of snow and ice, which also affects the nutrient supply of water, is determined by temperature. It has been stated that temperature is the most important factor affecting diatom growth, even more than food, and that phytoplankton abundance varies directly with it (91, 144, 45, 87, 4). However, Pearsall and Daily do not seem to think that temperature is of such primary importance (126). The optimum temperature for diatom development is dependent on the type of flora present (25, 87). It is the similarity of temperatures of the water of two regions rather than the latitude or altitude which is often the important factor in determining the type of flora present. Some species, such as *Anomoeoneis serians* v. *brachysira, Pinnularia streptoraphe* and *Cymbella gracilis,* prefer cold water. Others, such as *Cyclotella meneghiniana, Melosira granulata* and *Melosira varians,* have their maximum development in warm water (148, 136, 25, 94).

In general, diatom distribution seems to be more closely correlated with the chemistry of the water and temperature than with altitude. However, Hustedt found in the Dutch East Indies that at 1,500 m. standing water still had a tropical flora, while at 2,000 m. the flora was more temperate in relationship, though a few tropical forms were still plentiful. In flowing water the tropical character remained to 1,900–2,000 m., but above 2,500 m. it had practically disappeared. This was true of thermal as well as ordinary water. Altitude also seems to affect diatom structure in that at higher altitudes the striation of diatoms becomes finer (82, 149).

Summary. Thus it can be seen that there are a great many chemical and physical factors which may limit diatom growth, and that it is a balance between these factors which determines whether conditions are suitable to diatom growth and the type of diatom flora present. It has been found that various species of diatoms are specific for one or another factor. For these reasons diatoms have been very useful in helping to interpret prehistoric water and climate conditions. The greatest contributions in this field have been made by the Scandinavian and Finnish workers.

Geographical Factors

General. It is very difficult, if not impossible, to separate geographic from ecological barriers, for the two overlap and it is hard to tell whether one or both are affecting distribution. Certainly the geographic barriers do not seem to be so confining as in higher plants, for we do not have so much endemism. Also in many cases in higher plants it is difficult to know whether certain seeds never reached a given habitat or whether conditions were unsuitable for their survival if they did reach it.

As Kolbe states, so little is known about the diatoms in certain regions of the world that it is difficult to draw any general conclusions concerning the geographic distribution of diatoms. Also in many cases identification of diatoms has been made by people unfamiliar with the literature. Thus new species have been misidentified or overlooked (94). These facts would tend perhaps to make diatoms appear to have less endemism than they really do.

Though the statement is sometimes made that diatom species are cosmopolitan in their geographic distribution, it is not supported by the work of diatomists. Cleve (36) states, "Many diatoms are cosmopolitan, occurring in all parts of the world, but there are on the other hand many species, genera and groups which occur in only certain seas and climates". Boyer found that the diatoms of eastern and western North America are quite distinct, and that the Mississippi Valley region is more or less intermediate.

South America. In South America the degree of endemism seems to vary according to the region investigated; in South Chile 10–11% were endemic (103), in the Rio de La Plata 20% were endemic (58). In Lake Titicaca, of the 118 diatoms which were found, 38 were determined as characteristic of the flora and four were new (57). Frenguelli has written numerous papers on the fresh-water diatoms in South America, but much more work needs to be done before any general conclusions can be reached as to patterns of distribution.

Asia. In Asia the amount of endemism seems to be somewhat greater. In Siberia in Hanka Lake, Primorsk Government, 43% of the diatoms were endemic, while in Baikal Lake various species listed have shown endemism to be between 33% and 60% (154, 166). In North Manchuria in a stream in Khingan 19% were new (152). In the river Imen-gol near Hailar in northeastern Mongolia 9% were new (153). In Pin-Chiang-Sheng, Manchoukuo, the subaerial diatoms were found to have a large number of species in common with Europe (168). However, of the 59 species listed in this flora, 32% were new. In the Argun River only 5% were new (170). In ponds from Tientsin, North China, 30% were new, while in ponds from Peking 11% were new (151, 161). In Japan there is quite a variation in amount of endemism: in Ikeda Lake, Satsuma Province, Kiusiu Island, 14% (165); in a lake at Suriori near Seoul, Korea, 15% (156); in Biwa Lake, Honshu Island, 25% (164); and in Kizaki Lake, Honshu Island, 31% (163). In Fukien Province, South China (158) at Kuliang in an alpine stream, 20% were endemic (158), while at Amoy 12% were new (155). Patrick found in her studies of Siam and the Federated Malay States that 22% of the species were confined to eastern Asia and the Tibet region (124). Further study showed that of the species in this study only 33% are found on all continents, but that 60% are well distributed in the northern hemisphere. Seventy per cent of the species have been found in eastern United States, while only 28% have been located in western United States. These figures are no doubt exaggerated, since more work has been done in eastern than in western United States.

No very thorough study has yet been made concerning the distribution of diatoms in India. Of 56 species and varieties from Calcutta identified by Skvortzow 46% were new. In Assam the diatom flora of Loktak Lake, Mampur, was found to be Malayan in character (20).

At Ceylon (159) the diatom flora is tropical in character and very similar to that of India, Sumatra, Java and the Malayan region. However, very little study has been made of this region.

In the Sundai Islands 35% of the flora studied by Hustedt was endemic (86, 87). Of these about 60% characteristically live in running water, while 37% are typical of quiet water. From this Hustedt concluded that the most endemic flora is that characteristic of running water. Of the 645 species considered 56% were cosmopolitan in distribution. No doubt further studies in tropical regions may extend the range of some of the endemic species.

Though various species lists have been compiled of diatoms in Africa, our knowledge is still so fragmentary that no general conclusions can be drawn as to the geographic distribution of species.

Europe. Europe is perhaps the best known region in the world with respect to diatom flora. Though most of the species there are also found elsewhere, there are many of the alpine and northern regions which so far have not been noted in other geographic regions. As might be expected the flora of western Europe is most similar to that of eastern United States.

North America. As for North America there seems to be considerable difference between the diatom floras of eastern and western United States. Boyer (21) states that the Mississippi Valley region is intermediate as to its flora between that of eastern and western United States. Patrick (124) has shown that there is a greater similarity between the diatom flora of eastern United States and eastern Asia than between western United States and eastern Asia. As stated above, there is a great similarity between the floras of eastern North America and western Europe.

To date much more thorough work has been done on the distribution of marine diatoms than on the distribution of fresh-water and aerophil forms. No doubt further studies will extend some distributions and reveal other endemic species.

GENERAL CONCLUSIONS

From the foregoing studies it is evident that many factors influence the distribution of diatoms, and that many species are very specific as to their requirements. Their distribution seems to be restricted or made possible mainly by the pattern of ecological factors existing in a given region. Much is yet to be learned concerning exact requirements for growth of many species of diatoms. There is evidence that trace elements and organic growth substances may be more important than some of the more commonly recognized nutrient salts. As compared with higher plants many more species have very broad distribution patterns, and this has led to the belief of some that diatoms are generally cosmopolitan. However, this is not true, and the amount of endemism, as seen from a review of the literature, ranges from 10 to 30%. Just how accurate this figure is, it is hard to say. As yet our knowledge of diatoms in some parts of the world is very limited. Also, as pointed out by Kolbe, Hustedt and others, the smaller forms, which constitute an important part of the flora, are often overlooked.

It is interesting to speculate as to why so many species are so widely distributed geographically though definitely limited to certain types of ecological habitats. The most common reason given is that they are carried about by wind, birds and insects. Indeed all three are no doubt important agents in carrying diatoms for relatively short distances. We are able to trace some diatoms step by step in their distribution around the world. However, other diatoms have very discontinuous distribution patterns which are difficult to explain. In some cases further study may show a continuous distribution. So little research has been done on the ability of diatoms to withstand desiccation while being carried by wind or other agencies, that we do not know that even if they are transported for long distances they will survive. In many diatoms no resting spore or cyst is known.

Perhaps some factors which cause the wide distribution of diatom species as compared with many other plants lie in the physiology of the diatom itself.

All diatoms reproduce very rapidly by asexual means. Every time a diatom divides, a new plant is produced, whereas in multicellular organisms it takes many cell divisions to form a new plant. Thus a diatom in a new location very quickly surrounds itself with others just like it. Therefore, its chance of survival is increased. In only a very few diatoms is sexual reproduction definitely known, wherein either two organisms fuse and exchange nuclei or two sexual gametes unite. They belong to genera of the Pennales, *viz., Achnanthes, Anomoeoneis, Brebissonia, Cocconeis, Cymbella, Cystopleura, Dickieia, Eunotia, Gomphonema, Navicula, Nitzschia, Pleurosigma, Rhoicosphenia* and *Surirella.* The existence of sexuality in the Centrales still remains to be proved.

These very factors in themselves would greatly reduce the rate of speciation. First, the formation of new biotypes through different genic combinations, which is made possible by the process of meiosis and subsequent chance gametic combinations, would not occur. Second, the accumulation of both chromosomal and point mutations made possible in sexually reproducing forms by combination of gametes would not occur. The accumulation of mutations would occur only by subsequent mutations. Third, the drift toward homozygosity in gametic combinations which takes place when small portions of a sexual reproducing population is isolated would not occur in an asexual reproducing organism. However, any tendency toward homozygosity due to the effect of chance selection upon the organism would be the same. Thus some of the important factors in species formation would be eliminated.

In line with this reasoning is the fact that the genus *Nitzschia,* in which sexual reproduction has been found in several species, has the largest number of species of any diatom genus of the same geological age.

Thus the above mentioned factors may in part be the reason why portions of a species when isolated do not diverge from the type so rapidly as in sexually reproducing forms. The species would tend to remain constant and have a wider geographic distribution. In sexual organisms, once a segment of the population is isolated the operation of these factors help to bring about changes in the biotypes of a population, and thus in a given length of time speciation would occur faster and lead to a higher rate of endemism, other things being equal. In asexually reproducing organisms, once an organism survived it would speciate much slower. It is interesting to note that the Myxophyceae, which are completely asexual organisms, also have wide distribution patterns. Some of the other algae which have only sexual reproduction do not have such wide distribution of species. I believe, however, that in both plants and animals these groups which have only asexual or a high ratio of asexual to sexual reproduction tend to have more species with wider geographic distribution than strictly sexually reproducing forms. Of course there are also many other factors which are important in affecting distribution, and the above mentioned factors are only a few of them.

In diatoms another important factor is the medium in which they live. As regards certain ecological factors, such as temperature, water is more uniform than air, and an organism is not subject to such rigorous change. Also a plant living in water must become adapted to only one environment, while one living on land has two, air and soil. Thus the chance of survival in the former might be greater.

It is an interesting fact that a considerable number of diatom species found in the Miocene are still living today. This fact would also point toward the stability of species in diatoms. Much more work needs to be done on the evolution of diatoms as evidenced by the paleobotanical records. To date no critical work based upon the study of specimens has been made.

From this review it is evident that much more research concerning the ecological and geographical factors affecting distribution is needed. Such research will produce information of value to the plant geographer, ecologist and geologist.

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THE SPIKELET IN CYPERACEAE

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INTRODUCTION

The following paper was written after I had prepared a revised account of the Cyperaceae of the Malay Peninsula. I can not claim first hand acquaintance with Cyperaceae of other regions, nor to have consulted all the literature. I have, however, been impressed with the need to investigate more fully the tropical members of the family, and I have been led to the conclusion that the Hypolytreae (of Bentham) are the most primitive element in Cyperaceae. I have argued this thesis below and have given a general survey of the inter-relationships of the spikelet-form in the various tribes.

I suggest that here, as in many other families of plants, the tropical members are, on the whole, the most primitive, and a full understanding of them is, therefore, of great importance in a comparative morphological study of the family. Such a full understanding is still lacking, and I would commend the subject to anyone who may be interested. It is a small example of the wealth of significant information waiting to be extracted from the astounding variety of plant life in the tropics.

In Cyperaceae there are five main spikelet-forms:

1. Hypolytreae (of Bentham, not of Pax). Within each glume an arrangement usually considered as a partial inflorescence, by Bentham ("Genera Plantarum") as a flower, consisting of a terminal ovary and a varying number of scales around it, some or all of them with a single axillary stamen, the scales more or less distinctly arranged in whorls of 3 (usually 1–4 whorls), the two lateral scales of the outer whorl keeled and sometimes more or less completely joined laterally.

2. Scirpeae and Rhynchosporeae. Spikelets with many or few flower-bearing glumes, each glume enclosing a hermaphrodite flower consisting at fullest development of 6 (rarely to 10) perianth-segments or bristles, 3 stamens and an ovary with 3 stigmas. Various reductions from this condition occur, and some flowers may be male only; in some cases a spikelet may have only a single apparently terminal flower.

3. Sclerieae. Flowers unisexual. Some spikelets with a single female flower in the axil of a subterminal glume, the few glumes above it male or empty; other spikelets with several glumes enclosing male flowers only, of 3–1 stamens each.

4. Cryptangieae. Flowers unisexual. Spikelets in groups, with terminal female spikelet having a single terminal flower, and male spikelets in the axils of bracts below it; stamens 1–2, rarely 3.

5. Cariceae. Flowers unisexual. In *Schoenoxiphium* spikelets with basal female flower and several male flowers above it; in *Carex* the spikelets reduced to either the female flower or the male flowers, the female flower enclosed in a flask-shaped bract.

HYPOLYTREAE AND SCIRPEAE

The usual interpretation of floral structure in the family is to regard No. 2 in the foregoing as primitive and nearest the typical trimerous monocotyledon-form; the other flower structures are then considered to be derived from this. The structure within each glume of Hypolytreae (No. 1) is then regarded as homologous with a whole spikelet of condition No. 2. Such a view implies that in the Hypolytreae the terminal ovary and the scales, each with its one stamen, all represent separate flowers which are reduced from the trimerous hermaphrodite condition found in *Scirpus*.

But, as Bentham pointed out (1), the spikelets of Hypolytreae are in general structure and appearance very like those of *Scirpus;* and it is surely possible that the spikelets of the two tribes are homologous, the individual flower of *Scirpus* being homologous with the partial inflorescence (which Bentham called a flower) in the axil of each glume of Hypolytreae. If such were the case, the condition of Hypolytreae might be primitive, the flower of *Scirpus* reduced. The Hypolytreae are mainly plants of tropical forests, whereas the Scirpeae have become adapted to varying habitats in the open, in all parts of the world. Where related groups of plants have this difference of habitat and dispersal, it is often the group in the tropical forest which is more primitive.

The most important papers on the structure of the inflorescence in the Hypolytreae are by Goebel (7) and Bentham (1). Further evidence is presented by the figures in C. B. Clarke's "Illustrations of Cyperaceae" (4). The information given by Pax (10) is very indefinite, and, as pointed out by Goebel (9, p. 128), his diagram of *Mapania* is inaccurate.

The most highly developed condition of the inflorescence in Hypolytreae, apart from the peculiar South African *Chrysithrix,* is found in the monotypic genera *Scirpodendron* and *Lepironia,* both described by Goebel, the former in more detail. In *Scirpodendron* the apex of the whole spikelet is a female flower, *i.e.* an ovary, and below it are about ten spirally arranged scales, each with a single stamen in its axil, except the two scales next below the female flower which are usually but not always empty. In the axil of each glume of the spikelet is a similar arrangement of floral organs, with the following differences: the scales are approximately in alternate whorls of three instead of a spiral, and the two lateral scales of the outermost whorl are larger than the rest and strongly keeled, sometimes united at the base. The two keeled scales together perhaps represent the two-keeled prophyll which is normally present as the first leaf on all branches in Cyperaceae. The approximately three-ranked condition, most clearly shown by the outer whorls, is probably due to the bilaterally symmetric shape of the restricted space between glume and axis in which the scales develop; the two outer keeled scales having first been formed, the next would be formed half way between them (Goebel shows that this is actually the case), after which there would be gaps for an alternating whorl of three scales. According to Goebel's figures, the scales begin development a little earlier than the stamens in their axils, and the whole group of scales with stamens develops acropetally.

The number of scales with stamens in *Scirpodendron* varies considerably. Goebel found two to 11, with seven or eight most usual. The glumes immediately below the terminal partial inflorescence of the spikelet contain the fewest scales and sometimes may lack a female flower (ovary) altogether. On the other hand, the lowest glumes of a spikelet may have an abnormally large number of scales, all of them with stamens, with development of very reduced secondary inflorescences in the axils of the two keeled scales. Goebel also reports that there may be either two or three stigmas; and the large drupe-like fruit is variously six- to nine-lobed. Thus *Scirpodendron* gives the impression of being a primitive plant with the number of parts of the inflorescence and flower not fixed.

In the allied genus *Lepironia* the space between glume and axis is narrower, and Goebel found that stamens were developed only in the axils of scales on the side of the inflorescence towards the glume, the total number varying from one to five, while the scales were up to 15. One of Goebel's figures shows a slightly irregular arrangement of four whorls of three scales each, with no stamens in the axils of the inner two whorls, the third whorl with two stamens and the outermost with three. C. B. Clarke (4, t. 118) gives a diagram showing one stamen in the axil of each keeled scale; 12 scales, all with axillary stamens and almost in a single whorl; and then eight inner sterile scales around the female flower (ovary).

In the Malayan species of *Mapania,* the number of scales and stamens is reduced and the arrangement more regular than in *Scirpodendron.* There are only two whorls of three scales, the lateral scales of the outer whorl keeled as usual; stamens are present in the axils of all three scales of the outer whorl, but not of the inner whorl. In the closely allied *Paramapania* there are five or six scales (if five, the median one of the outer whorl is missing) and two stamens in the axils of the keeled scales, and three or four stigmas.

In some African species of *Mapania,* figured by Clarke (4, t. 108, 112–114), the arrangement is different. In *M. africana* Boeck. the scales of the outer whorl are connate, forming a tube within which are the three inner scales and three stamens, the stamens within the ring formed by the three inner scales. In *M. oblonga* Dur. & Schinz, *M. pycnocephala* Benth., *M. superba* Dur. & Sch. and *M. Mannii* Dur. & Sch. there are only two plus two scales, with a stamen in the axil of each outer scale.

In Asiatic species of *Hypolytrum* further reduction of parts has occurred, only the two keeled scales, each with an axillary stamen, being left on each side of the ovary. But in species of *Hypolytrum* from tropical Africa and America, as figured by Clarke (4, t. 103, 104, 107), the structure is more complex. In *H. pungens* Kunth, *H. strictum* Kunth, *H. africanum* Nees, *H. micranthum* Benth. and *H. Jenmanii* Clarke the two scales are joined together to form a cup or tube, shown in the diagrams as more or less convoluted, as if it might be partly formed from other scales. Within this cup are three or four stamens in *H. pungens,* three in *H. africanum* and two in the other species. In *H. supervacuum* Clarke (4, t. 105) the two keeled scales are separate, and a third scale, completing the whorl, is also sometimes present.

The illustrations and diagrams of the tropical American genus *Diplasia,* given by Clarke and Bentham, differ as to the inner scales, but otherwise agree and show a very interesting condition. The two outer keeled scales are present, without stamens, and apparently without the third member of the outer whorl. The inner scales are shown by Bentham as about six, joined together to form a tube, which encloses five stamens round the ovary. Clarke, however, shows only two inner scales, that towards the axis much broader than that towards the glume, and the two are not joined, though they are so close as to form in effect a tube, within which are eight stamens and the ovary. Uittien, in Pulle's "Flora of Surinam", describes the flower of *Diplasia* as having four scales, as in Clarke's illustration, and seven or eight stamens within the inner scales. The plants are very large, with stem and leaves to 300 cm. long and a compound umbel.

In the Australian genus *Chorisandra* each flower (4, t. 119) has 15 scales, but the outer lateral scales seem to be lacking. The outer scales are shown in whorls of three in *C. sphaerocephala* R.Br., the inner scales irregularly arranged; in *C. cymbaria* R.Br. the scales appear to be all spirally arranged. Seven stamens are shown in the former species, nine in the latter. It appears that there is room for a spiral development of parts within the glumes of this genus.

In *Chrysithrix,* a small African genus, there are many narrow scales, some or all with axillary stamens, and three large inner sterile scales round the ovary. This genus shows a possible ancestral condition of *Eriophorum* which has many narrow perianth-members.

The genera *Hypolytrum* and *Diplasia,* therefore, show that in the tribe Hypolytreae there could have developed a structure like a perianth, formed by the outer or the inner scales or both, completely surrounding the stamens which are arranged in a whorl or whorls round the ovary. This is in effect the condition of *Scirpus* and allied genera, though differing in detail and variable in the number of scales and parts concerned.

When we come to examine *Scirpus* and its allies we find that though six perianth-members of bristle-like shape are most common, there are many deviations from this standard. In *Eleocharis* two species are shown by Clarke as having eight bristles (*E. sphacelata* and *E. geniculata*), one of them apparently also with the rudiment of a ninth bristle; no floral diagrams of these are given. In other species five bristles instead of six may occur. For *Rhynchospora alba* Vahl, Clarke shows eight or ten bristles (4, t. 72), and in his floral diagrams indicates that he considers those above six to represent sterile stamens.

Scirpus, as now usually interpreted, includes a rather heterogeneous collection of species which remain after the more distinct genera *Fimbristylis, Eleocharis* and *Bulbostylis* have been segregated. I have no critical knowledge of the genus as a whole; the following notes are based mainly on Clarke's illustrations. The number of perianth-bristles varies from zero to eight in different species. In nearly all cases the bristles, of whatever number, are much of the same form, very slender with short stiff reflexed marginal hairs, but in at least three species they are different. In *S. membranaceus* Thunb. from South Africa (4, t. 47, 48) there are normally no bristles, but in some specimens there are two keeled lateral scales in the position of the scales of *Hypolytrum;* and in t. 48, figs. 1–4, the keeled scales are shown joined laterally to smaller scales which are interpreted as sterile stamens. Bentham (2, Vol. 3: 1051) suggests that this species indicates a connection between *Scirpus* and *Hypolytrum.* Clarke regards the keeled scales as "analogous to the broad scale-like hypogynous setae sometimes developed in *S. littoralis* Schrad" (5); he considers *S. membranaceus* to be allied to *S. littoralis.*

In *S. littoralis* (4, t. 50) there are four to seven perianth-members, varying much in width in different specimens, in some cases very narrow, in others spatulate and petal-like, all fringed with short hairs. The lateral outermost members, in the position of the keeled scales of *S. membranaceus,* are widest. Where seven perianth members occur, they are interpreted by Clarke as consisting of three of the outer whorl, two (lateral) of the inner whorl, with two sterile stamens (on the axis side of the ovary).

In *S. isolepis* Boeck. (4, t. 52, 14–17) there are two broad lateral scales in the position of those in *Hypolytrum,* but not keeled, and one stamen, not median, on the glume side of the flower. Pax includes this species in *Hemicarpha,* but Bentham restricts the latter genus to species with a single small median scale, considering them allied to *Lipocarpha.*

As regards number of stamens in the Scirpeae, three is usual, but two or one may occur. Pax interprets the three stamens as representing the outer of the two whorls of the liliaceous type; but C. B. Clarke invariably shows them as one of the outer whorl and two of the inner. Kükenthal states that in *Cyperus* the three stamens represent the outer whorl, but his diagram shows them asymmetrically placed, as in Clarke's diagrams. It is certain that the three stamens are always on the side of the ovary away from the axis, *i.e.*, between glume and ovary. I have examined young spikelets of *S. mucronatus* and find that the lateral stamens are not opposite perianth-bristles, but apparently between them. It seems at least very unlikely that the three stamens represent a symmetrical whorl, as postulated by Pax.

The interpretation of the flower of *Scirpus* and allied genera according to an arrangement of two whorls of perianth and two of stamens is thus not clearly established, and I suggest that an interpretation as a reduction from the more complex condition of *Scirpodendron* is equally possible and explains both the peculiarities and irregularities found in *Scirpus.* It is clear from the condition of *Diplasia,* indicated less clearly also in *Hypolytrum pungens,* that some of the inner stamens of the *Scirpodendron* arrangement may persist while their scales are aborted. It is also clear from many examples that the stamen in the axil of any scale may be aborted, the scale persisting; in such cases it is usually the stamens on the side of the ovary towards the glume which persist. *Diplasia* shows that a perianth of two whorls may exist, with all stamens within the inner whorl. We have thus all the necessary possibilities for deriving a *Scirpus* flower from the potential 12 scales and 12 stamens of *Scirpodendron.*

Bentham and Pax both recognized a relationship between *Scirpus* and *Hypolytrum;* Bentham evidently considered the "flower" of Hypolytreae as homologous with that of *Scirpus,* though he did not suggest that the Hypolytreae represented the primitive condition. Pax, on the other hand, made a most confused arrangement. Apparently knowing only the most reduced condition of *Hypolytrum,* he placed it in the subfamily Scirpoideae, while the remaining Hypolytreae of Bentham he placed in his subfamily Caricoideae. He regarded *Hypolytrum* as a very primitive type, with two prophylls (the two keeled scales) but with a reduced number of stamens and no perianth; and he placed it as the most primitive member of the subfamily Scirpoideae, most of the remaining members of which he considered to have lost their prophylls. He did not explain the nature of a prophyll and evidently had no clear idea on the subject, as he placed *Lipocarpha* in the same tribe as *Hypolytrum* on account of its two median scales, which are quite different in appearance and position from those of *Hypolytrum*.

In view of the diversity of reduction-forms found in the Hypolytreae, it seems quite possible that flowers of the *Scirpus* type might arise on more than one evolutionary line, and possibly some of the subgenera of *Scirpus,* especially that containing *S. isolepis,* may represent such lines. Studies of floral development may throw light on this question; but an investigation of dried mature flowers can hardly show the homologies of the parts with sufficient clearness. If the derivation from *Scirpodendron* is a true one, there are many more possibilities to be considered than in a derivation from the formula P 3 + 3, A 3 + 3, and the interpretation therefore more difficult.

A derivation from some such ancestor as *Scirpodendron* involves also more fundamental considerations. It means that the trimerous arrangement of the flower, perhaps apart from the ovary, in Cyperaceae originated *de novo,* owing to the shape of the restricted space in which the flowers develop; and that the trimerous arrangement, therefore, can not be invoked as evidence of relationship with other families of trimerous monocotyledons unless the latter are considered to have had a common ancestor with Cyperaceae or to have originated from it.

Assuming the *Scirpodendron* condition to be primitive, it seems likely that the nearest relations of Cyperaceae are the families of the Pandanales (Pandanaceae, Typhaceae, Sparganiaceae), though the relationship does not appear very close, perhaps partly because of the unisexual inflorescences of the Pandanales. But in *Freycinetia* the male flowers may have an ovary-rudiment, and in the female flowers "an irregular number of staminodes usually surrounds the base of the pistil" (Rendle); perhaps, therefore, the ancestors of Pandanaceae had bisexual flowers or inflorescences.

In *Pandanus* each male flower consists of an indefinite number of stamens without bracteoles or perianth of any kind. In *Typha* the male flower consists of three stamens, with an irregular arrangement of scales below them. If we imagine a flower with a terminal ovary, stamens attached to the axis below it, and scales at the base, as in *Typha,* we have a possible ancestral form from which the flower of *Scirpodendron* may have been derived. The additional scales of *Scirpodendron* might have been formed by sterilization of some of the stamens. If such flowers were arranged on an axis, one terminal and the others each in the axil of a bract, we should have the prototype of a spikelet. As the inflorescence became condensed to spikelet-form with overlapping bracts, the space for the development of all organs would have become limited, and a definite arrangement of such organs would necessarily have followed.

The stamens in Pandanales all have a more or less developed sterile apex of the connective. This occurs also in Cyperaceae and in other monocotyledons; but it may possibly be a link between Cyperaceae and Pandanales.

As regards ovary-structure, the condition of Cyperaceae is unstable and again suggests that the family was not derived from a stock which normally had three carpels. In *Scirpodendron costatum* the fruit is a drupe and has six to nine longitudinal ridges; the stigmas may be two or three. In *Mapania* also the fruit is a drupe, but in most members of the family it is a nut; in many genera some species have two stigmas and some three (the nut biconvex or three-angled to correspond), and in *Fimbristylis globulosa* flowers with two or three stigmas may occur in the same spikelet (this probably occurs also in other members of the family). When the nut is biconvex, its edge or its face may be towards the axis. In the genus *Evandra* (Australian), of the tribe Rhynchosporeae, there are eight stigmas, the nut with several longitudinal grooves (*Evandra* is also peculiar in having 12–20 stamens). In *Paramapania* there are three or four stigmas. In *Pandanus* there are drupaceous syncarps formed by the union of a variable number of monocarpellary ovaries, each with a single ovule. It is hardly likely that the *Scirpodendron* fruit was derived from *Pandanus;* but the fact of *Pandanus* having a drupaceous fruit, its units formed of a variable number of carpels, is perhaps an indication of relationship between *Pandanus* and the primitive members of Cyperaceae.

Another resemblance between Cyperaceae and *Pandanus* is the way in which the foliage leaves grade into the primary bracts of the inflorescence which is terminal. This gradation occurs in the majority of the Cyperaceae, including *Scirpodendron, Hypolytrum* and one section of the genus *Mapania.* The leaf-form of *Pandanus* is also closely matched by that of *Scirpodendron* and *Mapania,* and occurs in a less pronounced way in other tribes of Cyperaceae where the leaves are broad enough, *e.g.,* in *Cyperus diffusus* and *Scleria corymbosa.* Mr. Corner has, however, examined the anatomy of *Pandanus* and *Mapania* leaves and informs me (verbally) that the two are very different in structure; but a very close relationship between *Pandanus* and *Mapania* is not claimed, and it seems possible that a resemblance of external form might persist with changes of anatomy. I have myself examined the anatomy of *Scirpodendron* leaves and find it essentially similar to that of *Pandanus* leaves.

Such a suggestion of relationship between Cyperaceae and the Pandanales is only a tentative one; but if *Scirpodendron* is really a primitive type in Cyperaceae this relationship seems more likely than any other, and a comparative morphological study of the plants concerned, with this idea in mind, would be worth undertaking.

The family Juncaceae is sometimes associated with Cyperaceae. It is so considered by Hutchinson (8) and by Fritsch (6). In such cases Cyperaceae is usually regarded as being reduced from the condition of Juncaceae, and Juncaceae from the Liliiflorae. But if the argument here presented is correct, Juncaceae must be either an unrelated group or derived from Cyperaceae; and conceivably the primitive Liliiflorae might also have evolved from the primitive Cyperaceae.

RHYNCHOSPOREAE

This tribe was placed in the same subfamily as Scirpeae by both Bentham and C. B. Clarke, and is so placed again by Mattfeld in the later editions of Engler's "Syllabus"; but Pax in his "Pflanzenfamilien" synopsis of Cyperaceae removed Rhynchosporeae to the subfamily Caricoideae on account of a supposed sympodial structure of the spikelet. Pax states that in Rhynchosporeae the apparent lowest flower in the spikelet is terminal and that subsequent flowers are each borne on a new lateral axis arising in the axil of the last glume below the preceding flower. He gives no detailed descriptions nor drawings of any such case, and only floral diagrams of *Asterochaete, Elynanthus* and *Gahnia.* In these diagrams he indicates the presence of a two-keeled prophyll accompanying the second flower. Such a prophyll is normally present at the base of every branch in Cyperaceae and would be clear indication that branching had occurred. I have not examined *Asterochaete* or *Elynanthus,* nor are they figured by Clarke; but in *Gahnia* I can not detect the presence of such a prophyll, nor is it indicated in any of Clarke's illustrations of *Gahnia.* Pax states that the spikelets of *Rhynchospora, Schoenus* and *Cladium* are also sympodial, but gives no drawings and makes no specific reference to prophylls. *Rhynchospora* and *Cladium* have small spikelets which are difficult to investigate; but *Schoenus calostachyus* has large spikelets which I have examined, and I find no indication whatever of a prophyll. The base of each glume in *Schoenus* is decurrent on the sides of the rachilla down to the level of the preceding flower. Apparently Celakowsky interpreted this to mean that the glume is connate with a shoot in its axil (*cf.* 11, fig. 117J) ; but such is surely an unnecessary assumption, and in the absence of a prophyll there seems no evidence that branching has occurred. Moreover, the condition is precisely similar to that common, but not universal, in *Cyperus,* where the base of each glume is decurrent as a wing on the next lower internode of the rachilla; but nobody has suggested that the rachilla of *Cyperus* is sympodial. The decurrent base of the glume is probably a consequence of the elongation of the internode on a two-ranked spikelet in both *Schoenus* and *Cyperus.* In *Scirpus, Fimbristylis, etc.,* with closely placed scales in several spirals, the base of the glume is not decurrent, but forms a little cup when the lamina has fallen.

Rhynchosporeae are distinct from Scirpeae in the predominantly panicled inflorescence of small spikelets with few flowers and the presence of several sterile glumes at the base of each spikelet. But a panicled inflorescence occurs in *Fuirena,* and condensed inflorescences in *Rhynchospora* and other genera; the section Kyllinga of *Cyperus* has only one or two flowers, and some species of *Schoenus* have several flowers. It is thus difficult to frame a definition of Rhynchosporeae which will separate it sharply from Scirpeae, and there can be little doubt that the two are closely allied. Several genera of Rhynchosporeae are much specialized, notably *Oreobolus* (small size of plants, single apical flower, persistent flattened perianth -members), *Remirea* (single flower, apparently apical and enclosed in a peculiar glume which is thick and corky when mature, surrounding the nut) and *Gahnia* (single hermaphrodite flower, apparently terminal, with or without a male flower, the nuts suspended after ripening by the stamen-filaments which are attached apically to the glumes). Rhynchosporeae probably represent a series of developments on different lines from the condition of *Scirpus,* rather than a coherent tribe.

THE TRIBES WITH INVARIABLY UNISEXUAL FLOWERS

These tribes are: Cryptangieae, almost exclusively American, including several genera but all with few species, mostly small plants; Sclerieae, consisting chiefly of the large tropical genus *Scleria;* and Cariceae, consisting chiefly of the very large, worldwide, but mainly temperate, genus *Carex*. On any interpretation these are specialized genera, and the most remarkable fact is that *Carex,* one of the most specialized of all, is yet the largest genus of the entire family Cyperaceae and worldwide in range. If a late development of the family has attained such a diversity and range of distribution, the family itself must surely be an old one.

J. Mattfeld, in Engler's "Syllabus" (9), still includes *Mapania* and its allies in the division of Cyperaceae with unisexual flowers. This is a formally correct arrangement on the interpretation of the "flower" of *Mapania* as a partial inflorescence; but it is here argued that this partial inflorescence is the primitive form which gave rise to the flower of *Scirpus,* and is therefore functionally a hermaphrodite flower.

Cryptangieae are represented in Malaya only by *Diplacrum.* This was included in *Scleria* by Bentham, and considered very closely allied to *Scleria* by Clarke, but Goebel (7, pl. 15) has shown that the spikelet-arrangement corresponds to that of the other genera of Cryptangiae. *Diplacrum* has species in tropical America, Africa and Asia, whereas the other genera of Cryptangieae are exclusively American.

In the Cryptangieae each branch of the inflorescence bears a series of small bracts, usually in a spiral arrangement; the basal bracts have small axillary male spikelets, the upper bracts (two in Diplacrum) are without spikelets, and a female flower occupies the apex of the branch, surrounded by the sterile inner bracts which might be regarded as empty basal glumes of a terminal female spikelet. Such an arrangement might have been developed from a spikelet-group of one of the Rhynchosporeae by loss of the ovaries in the flowers of the lower spikelets and retention of a terminal spikelet with terminal female flower.

It appears that the genera of Cryptangieae are not very closely allied, and they may represent more than one line of evolution. As regards *Diplacrum,* its nut suggests a relationship to *Scleria.* Its differences from *Scleria* lie in the terminal position of the female spikelet and the lack of empty glumes above the female flower, which appears to be terminal. In *Scleria* the female spikelets are usually axillary at the bases of branches or branchlets which bear male spikelets higher and terminally; and in each female spikelet the axis extends above the flower and bears one or more small glumes, so that the female flower is not terminal. But there are cases in *Scleria* in which the female spikelet is terminal; and the case of *S. flaccida,* illustrated by Clarke (4, t. 127, fig. 4) with a terminal female spikelet and several lateral male ones below it, is very near *Diplacrum.* It seems likely, therefore, that *Diplacrum* is an offshoot of *Scleria.*

Pax placed the Cryptangieae (his Hippiinae) and the Hypolytreae (his Chrysitrichinae) as subtribes of the tribe Hippieae, considering them related because in each there is a terminal female flower, with bract-like organs below it, in the Cryptangieae with a male spikelet in each bract, in the Hypolytreae a single stamen. But these two arrangements are very different, and there is nothing in Cryptangieae resembling the massive spikelets of the more primitive Hypolytreae. The association of the two groups appears to me to be entirely unnatural.

Sclerieae. *Scleria* could be derived from the condition of *Cladium* which has small spikelets with the upper flowers male. From such a condition it needs only that the hermaphrodite lower flower should lose its stamens to produce a spikelet like that of the subgenus Hypoporum (*S. lithosperma, S. corymbosa* and allies). The further development to Eu-scleria, in which the spikelets are either male with several flowers, or female with one flower and a few empty glumes above it, is not a very long step.

The nature of the disc in *Scleria* is not discussed in any work that I have seen. It is always three-lobed and looks like a development from three members of the perianth of Scirpeae, but if so is much modified by thickening of the members.

Cariceae. The homologies of the parts of the spikelets in this tribe have been much discussed, and there is general agreement on the subject. I think, however, that the significance of the utricle as a prophyll is not sufficiently emphasized. Below is a brief statement on the matter.

In *Schoenoxiphium* each spikelet consists of a basal female flower with several male flowers above it. The female flower and part of the axis of the spikelet above it are almost enclosed in a flask-shaped bract called an "utricle". This bract is actually the prophyll or first bract at the base of the spikelet, which in most Cyperaceae is empty. In *Kobresia* the arrangement is similar, but the spikelet-axis above the female flower is very short, and wholly male spikelets may be present. In *Uncinia* the male flowers are all in a terminal spikelet, the female spikelets laterally below, each reduced to a single female flower enclosed in an utricle but with the rachilla persisting above the flower and exserted from the utricle. *Carex* is like *Uncinia,* but the rachilla is lacking or rudimentary, and the female flower thus appears to be terminal on its axis.

In the subgenus Indocarex, which has many small spikelets and may perhaps represent the most primitive element in *Carex,* each spikelet consists of a male terminal portion with female flowers at the base. We may derive such a spikelet from a group of spikelets of *Schoenoxiphium,* one terminal and the others in the axils of small bracts below it; if the terminal spikelet loses its female flower and the lateral spikelets lose their terminal male portion entirely, the result is the condition of Indocarex. In the other sections of *Carex* the spikelets are generally fewer, larger and all except the terminal one entirely female.

The utricle of *Carex* and allied genera is clearly the glume in the axil of which the female flower develops. But it is unlike the normal glumes of Cyperaceae in being almost closed and in having two keels, usually with a cleft apex. In fact, it is very like the prophyll which is borne at the base of every lateral spikelet and every new branch in Cyperaceae, backing the axis from which the branch arises. Normally the prophyll at the base of a spikelet in Cyperaceae has no axillary flower.

Reverting to *Schoenoxiphium,* we see that its spikelet is like that of *Scleria,* subgenus Hypoporum, in having a single female flower with male flowers above it, and to that extent may be derivable from the condition of *Cladium*. But *Schoenoxiphium* is peculiar in having the female flower in the axil of the prophyll with male flowers in the axils of glumes immediately above it, whereas in *Scleria* and all the Rhynchosporeae there are several empty glumes above the prophyll. However, in *Scleria* the female spikelets are usually in the axils of prophylls at the base of short branches which terminate in male spikelets. If we could reduce the female spikelet to a single flower we should have the condition of *Schoenoxiphium*. But *Carex* can not have been evolved from the specialized genus *Scleria;* it was probably on another line from one of the Rhynchosporeae which developed female spikelets in the axils of prophylls.

SUMMARY

Summing up the foregoing comparative account of spikelet and flower structure, we have a primitive Hypolytreae, with various intermediates leading to Scirpeae, which have a more or less regular trimerous flower-structure with usually many-flowered spikelets, *Cyperus* with its elongate two-ranked spikelets being a special development; from Scirpeae possibly several lines lead to Rhynchosporeae, with mainly panicled inflorescences of small few-flowered spikelets, some flowers often male; from the Rhynchosporoid condition probably arose on different lines the genera of Cryptangieae, Sclerieae and Cariceae.

Bentham's division of the family (2) into two series, MONOCLINES (Scirpeae, Hypolytreae and Rhynchosporeae) and DICLINES (Sclerieae, Cryptangieae and Cariceae), thus can not be accepted as an indication that the tribes of Diclines are more nearly related to each other than to the Monoclines; according to the view here expressed they are in fact divergent from different origins in the monoclinous state. Bentham's arrangement is, however, a reasonable one if interpreted as a statement of evolutionary progression, except that the Hypolytreae should come at the beginning of the Monoclines, instead of after the Scripeae.

Probably no other arrangement of the genera and tribes is at present possible, though comparative study may indicate probable evolutionary lines. Little such comparative study appears to have been attempted, in contrast to the amount devoted to the Gramineae. There appears to be no modern comprehensive survey of the Cyperaceae. The only comprehensive monographs are those of

Kükenthal, which deal only with the specialized groups of *Cyperus* and *Carex*. Uittien's recent studies of Hypolytreae are valuable but do not consider fundamental questions of comparative morphology.

Subsequent to Bentham's arrangement in "Genera Plantarum", the principal new schemes proposed are those of Pax in Engler's "Pflanzenfamilien" (7), C. B. Clarke in his unpublished monograph, of which a summary was issued in 1908 (6), and of Mattfeld in Engler's "Syllabus". Pax's arrangement has been criticized above and is in my opinion very unsatisfactory.

C. B. Clarke studied Cyperaceae for more than 20 years and probably had a greater knowledge of the family than any other botanist, but his scheme is criticized by Kükenthal as being too artificial. It differs from Bentham's in having four subfamilies: Scirpo-Schoeneae (Bentham's Scirpeae and Rhynchosporeae), Mapaniae (Bentham's Hypolytreae with the addition of *Remirea,* which may be correct), Sclerieae (Bentham's Sclerieae and Cryptangieae) and Cariceae. The scheme is thus not greatly different from Bentham's, and I do not think it is any improvement. The union of *Scleria* with all Cryptangieae is perhaps doubtful. Unfortunately no commentary on the scheme by its author was published.

Mattfeld's scheme is a modification of Pax's differing mainly in the removal of Rhynchosporeae from Caricoideae to Scirpoideae. He thus corrects what I believe is one of Pax's major errors, but he leaves *Mapania* (presumably with allied genera, but they are not mentioned) in the same tribe as the Cryptangieae of Bentham, which I consider to be Pax's other major error; and he places the subfamily Caricoideae first, whereas (as typified by Carex) it should come last, being the most highly evolved.

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SOIL MICROORGANISMS AND THE RHIZOSPHERE[1]

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INTRODUCTION

In 1904 Hiltner (28) introduced the term "rhizosphere" to designate that portion of the soil which is subject to the influence of the plant root system, and noted that this soil supported greater microbial activity than soil more distant from the roots. This observation was confirmed subsequently by many investigators whose work up to 1929 has been thoroughly reviewed (93). The present article concerns studies carried out since that time.

Much of the work in this field has dealt with the physiology of the plants and their roots (carbon dioxide evolution, absorption of minerals, root excretions) in relation to their influence on the rhizosphere. It is intended here to emphasize the microbiological aspects of this phenomenon, considering root physiology only insofar as it is necessary to interpret the recorded activities of soil microorganisms in the rhizosphere. Reviews of root activity and physiology will be left for the qualified plant physiologist (68). However, as a background for the elucidation of the microbiological phenomena involved, the inclusive statement of Starkey (93) concerning the influence of higher plants on the rhizosphere may perhaps be appropriately quoted here: "Among the various modifications of the soil which the plants may provoke are the following: lowering the concentration of certain mineral nutrients in the soil due to their absorption, partial desiccation of the soil by absorption of water, increase in soil carbonates following root excretion of carbon dioxide, contribution of microbial foods by sloughed off root portions or excretions. Some of these effects may directly affect the soil population and others indirectly by modifications of the physical composition of the soil".

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√ The organisms in the rhizosphere in turn may exert profound influences upon the plant, which include the multiplicity of reactions carried out by soil microorganisms in general: liberation of plant foods by decomposition of soil organic matter and by the solvent action of carbon dioxide and other acids which they produce; transformation of nitrogen and sulfur compounds (ammonia, hydrogen sulfide) into readily available forms which may be beneficial or toxic to plants, affecting the oxygen tension in the immediate vicinity of the roots; competition with higher plants for available nitrogen and minerals; synthesis of vitamins and auxins; and entering with higher plants into various associations which may be beneficial or harmful (120, 133). In view of the increased density of organisms in the root zone it is to be expected that associative and antagonistic relationships will be particulary pronounced with resulting favorable or unfavorable effects on the plant (21, 122).

One of the most important considerations in the study of the microorganisms of the rhizosphere is obviously that concerned with the extent of the zone of root influence. It is to be expected that the effects of root growth and physiology will diminish with the distance from this organ, but whether the most characteristic picture of the microflora of the rhizosphere may be obtained from the soil adjacent to the root, from that on the intact root itself, or from root scrapings, is still controversial. Whereas in most cases soil at the immediate root surface is used, it has been suggested (105) that a more characteristic rhizosphere microflora is obtained by using macerated root tissue after the roots have been washed in several changes of sterile water. Various workers (24, 80) have made distinctions between the "outer" rhizosphere (soil adhering to the root) and the "inner" rhizosphere (root surface). The localization of microorganisms on roots and around root hairs, as demonstrated by the contact slide method (30, 33, 62, 99), supports the practise of utilizing the roots themselves in rhizosphere studies to obtain the most characteristic picture of the microflora.

In order to demonstrate a "rhizosphere effect" on numbers of microorganisms it is necessary to make comparative counts on soil more distant from the root. The extent to which the roots influence the soil microflora may then be expressed by the rhizosphere: soil (R: S) ratio, that is, the number of organisms in the rhizosphere soil divided by the number in the soil at a distance from the root (calculated on a dry weight basis). Counts of numbers on the root surface alone give ordinarily no indication of the influence of the plant and are of use mainly in comparing effects of different plants grown under identical conditions. Environmental factors may be such as to reduce numbers of organisms in both soil and rhizosphere (low temperature, excess moisture), but whether the "rhizosphere effect" is reduced or not can be determined only by means of rhizosphere and soil counts (41). For evaluating the influence of soil type, treatments and other factors on the root surface microflora, this ratio is of fundamental importance (41).

Comparative tests with control soils are particularly important in demonstrating a selective action of roots on definite types or groups of microorganisms. The mere isolation of species from the rhizosphere, as reported by various workers (80,83,115), is without significance in the absence of data from soil apart from the plant.

METHODS OF STUDY

The microflora of the rhizosphere may be studied by means of cultural methods or by direct microscopic examination. Since the former yield by far the most significant information, a general procedure utilizing them is described below.

Plants are carefully removed from the field or greenhouse pot and adherent soil gently broken to release the root system which is then shaken to remove superfluous soil and immersed in a known volume of sterile water in a weighed flask (10, 110*a*). After thorough shaking, suitable dilutions are made and the roots removed and washed, the wash water being collected in the original dilution flask. The moisture in the original flask is then driven off, the contents weighed and the number of organisms computed per gram oven-dry "rhizosphere soil", allowance being made for the amount of material removed in perparing dilutions.

There is some question as to whether or not the roots themselves should be used as the basis for calculation of numbers of organisms in the rhizosphere, although this would be impossible in comparisons of fibrous and fleshy roots. Clark (12) contends that an appreciable error may be involved in the use of rhizosphere soil for this purpose, especially where plants are obtained from soils of varying moisture content, since this would affect the quantity of soil adhering to the roots. As there are few comparative data on this point, it may be best to include dry weights of both roots and rhizosphere soil in any presentation of results.

It is preferable to use the complete root system where possible. However, when roots are bulky or particularly voluminous (mangels, legumes, *etc.*) representative portions may be used (110*b*). Samples of soil apart from the root should be studied concurrently, as it is only by this means that a "rhizosphere effect" can be demonstrated. The media and methods employed for estimating numbers and types of organisms in the rhizosphere will depend of course on the kinds of organisms under investigation.

The microscopic procedure involves either use of the contact slide technique of Rossi-Cholodny or direct examination of roots (30, 33, 59, 62, 99, 102, 112). The former involves burying glass slides or cover glasses in soil so that roots of plants come in contact with them, removing at suitable intervals and examining, usually after staining. As Starkey (99) states, "the method provides a means of obtaining a more accurate conception of the development of microorganisms in response to root growth, the types of organisms affected, the localization of the individuals and groups and the sequence of morphological types". However, as ordinarily conducted this procedure does not permit culturing of the organisms, nothing can be ascertained regarding their physiology, specific bacteria cannot be recognized on the basis of morphology alone, and, in addition, the sequence of development is problematical. Furthermore, as Linford (62) points out, contact slides do not retain nematodes, nor can protozoa be recognized; again, certain fungi present in abundance on roots do not adhere to slides. Many of the larger compact colonies are also removed with the root from the slide. By means of flooding these slides with selective media Isakova[4] (33) extended this procedure to permit the culturing of the adherent organisms.

Roots may also be removed and examined under the microscope (107) or observed *in situ* by means of specially constructed observation boxes (61, 62). The glass coverslips used in this procedure may later be stained. This method yields interesting

[4] The Russian literature was assembled by M. I. Timonin.

information concerning localization of various microbes of the soil on roots and root hairs but is subject in general to the same criticisms as the contact slide method.

QUANTITATIVE EFFECTS OF HIGHER PLANTS ON SOIL MICROORGANISMS

That bacteria, actinomycetes and fungi of the soil find the root zone a more congenial environment for development than soil apart from the root is now generally accepted. Very little has been done with the soil microfauna in this connection or with soil algae. Linford (60, 62) reported that protozoa and microphagous nematodes congregate and multiply in the root zone. By means of cultural methods, Katznelson (41) noted R: S ratios as high as 23 for protozoa on plants in manured soil. Algae have not been mentioned in studies on the rhizosphere until recently when it was reported (41) that a distinct effect occurred in the rhizospheres of mature but not young mangels in both fertilized and unfertilized soil.

As may be expected, numbers of organisms in the root zone are influenced by many factors: nature and age (or stage of growth) of plant; type, moisture content, reaction, and treatment of the soil. Frequently, it is impossible, however, to determine which of these phenomena is responsible for the observed effects. Soil treatment, for example, may stimulate plant growth which, in turn, may cause an increase in numbers of organisms on the root surface. An increase in number of organisms may be expected following certain soil treatments, but a "rhizosphere effect" need not necessarily result unless the plant itself is stimulated in some manner.

Nature and Age of Plant. Comparisons of rhizosphere counts of various plants grown under identical conditions are fairly numerous. These counts, however, are not always comparable, since they are usually obtained from plants of the same age but certainly not at the same stage of development because of different growth rates and maturity periods. Insofar as the major groups of soil microorganisms are concerned, different plants exert considerably different effects which are also influenced by different stages of growth (98). Starkey (93) noted that alfalfa roots stimulated filamentous fungi only slightly but that eggplant exerted an appreciable effect. The latter also caused greatest increases in actinomycetes and bacteria, whereas sugar beet and corn were the least effective. On comparing various other plants he (94) observed that numbers of actinomycetes and filamentous fungi were slightly higher on the roots. However, considerably greater increases of bacteria occurred, rape being particularly effective, potatoes least. In no case was the R: S ratio more than 3. This can be explained by the fact that the samples were not taken from the root but from the soil surrounding it, a procedure which undoubtedly diluted the numbers of organisms in the rhizosphere. In general, most of the plants did not exert a great effect at the early stage of growth, and the maximum effect generally appeared after the plant had attained appreciable size, had reached the limit of vegetative growth or had bloomed or started to degenerate. After death of the plant a rapid decline in numbers of bacteria occurred. Potatoes and oats particularly showed rapid increases in numbers after the early stage of growth and extensive decline after maturity and death. Biennials showed a marked effect, even in the later stages of growth. In later studies Starkey (96) reported much greater numbers of organisms in the rhizosphere, especially on the immediate plant root, and a marked decline with distance from it. Again the bacterial population was affected to a much greater extent than actinomycetes and filamentous fungi. Sweet clover exerted a profound effect, yielding an R: S ratio of 50 at 356 days and 217 at 437 days. Actinomycetes were stimulated to a maximum ratio of 8.6 by sweet clover at 437 days. Younger plants such as bean exerted a slight effect, mangel beets and corn none. Filamentous fungi were affected slightly more than actinomycetes; again sweet clover was most effective, yielding a ratio of 10 at 437 days. Of the first-year plants the greatest effect was obtained with corn (R: S ratio of 13). It appears then that greater numbers of microorganisms, especially bacteria, were found on roots of legumes, though non-legumes also exerted a distinct effect.

In a study of the microflora of the roots of a variety of crops Gräf (24) distinguished between the "inner" rhizosphere (root surface), the "outer" rhizosphere (soil adhering to the root), and soil distant from root (control). Monthly determinations indicated that total bacterial counts were as expected, highest on the root surface. A pronounced increase in numbers occurred at the root surface during the period of growth followed by a decline during the ripening period and a further decline after harvest. She noted too that the increase in total numbers was least in the case of grains and strongest with legumes and rape. Thom and Humfeld (107) found bacteria to be stimulated on roots of alfalfa, rye and vetch more than fungi, and fungi on the whole more than actinomycetes. In the same soil they found that alfalfa stimulated bacteria more than rye; in another soil rye was more effective than vetch. Fungi and actinomycetes were stimulated to an equal degree by alfalfa and rye in the first soil, but no rhizosphere effect on actinomycetes occurred with the second soil.

Krassilnikov *et al.* (58) noted that the rhizosphere of cultivated peanuts harbored from 10 to 100 times as many organisms as soil apart from the root; this condition persisted until the flowering stage. Towards maturity numbers of bacteria diminished, and those of fungi and actinomycetes began to increase. In studies with wheat, maize, sunflower and soya he and his co-workers (49, 56, 57) observed marked increases in total numbers of microorganisms in the rhizosphere. That of soya had greatest numbers, maize and sunflower less and wheat least. Counts were highest with sunflower and soybean during the early part of the growing period and in the later part of the season. Evidence of a distinct "rhizosphere effect" by roots of the tea-bush was obtained by Obraztzova (78). With age of plant there was an increase in the numbers of microbes on the roots; a two-year old plant yielded twice as many organisms as an old flowering bush. There was apparently no essential difference between the kind of microorganisms in the rhizosphere of the tea-bush or grass. Manzon (74), in a study of a variety of leguminous and non-leguminous plants, concluded that the numbers and activity of the bacterial population of the rhizosphere varied greatly, depending both on the species of the plant and on the phase of its development. Highest bacterial numbers were obtained with legumes.

In studies of sandy desert soils Sabinin and Minina (84) observed an extraordinary example of the influence of root systems on the soil microflora. Soil at a short distance from the root system appeared to be sterile. The microbial life of these soils develops only in conjunction with the root systems of the plants.

By means of the contact slide technique, Starkey (99) did not observe any striking differences between mangel beet, barley, maize, rape, vetch and soybean. The most obvious change, an increase in abundance of organisms, was noted as the plants matured. Clark and Thom (13) found that the total bacterial count on roots of cotton in treated or untreated soil increased from July to August, then decreased. However, since no R: S ratios were computed, it is not evident to what extent the plant itself influenced these counts. Clark (10) found that the rhizosphere populations in manured soil were generally greater than in untreated soil, though the effect of manuring was much more pronounced in soil apart from the root. In a further study (11) he found wheat roots to support somewhat larger numbers of bacteria than barley and oats. Total counts in the rhizospheres of beans, radishes, lettuce and corn were highest in this order, though for these crops the "rhizosphere effect" can not be estimated in the absence of data from control soils.

Adati (1) made an extensive study of numbers of bacteria, actinomycetes and fungi in the rhizospheres of different crops growing in sand, loam, clay and humus soils. Counts were given for control soil, for rhizosphere soil directly surrounding the roots and for soil at an unspecified distance from them. Much higher numbers of all organisms were found in the rhizosphere directly adjacent to the root, decreasing with distance from it. In general, numbers were highest on legume roots, a ratio of 197 for bacteria being obtained with peas. Bacteria were stimulated to the greatest extent, fungi usually next and actinomycetes least, although R: S ratios for this last group of organisms were in certain instances as high as 62. Ratios varying from 2 for oats to 45 for flax were reported in a study of a variety of crops (65). Timonin (111), on comparing the microbial populations of the rhizosphere of seedlings of wheat, oats, alfalfa and peas, found that bacteria and actinomycetes (together) were 7 to 71 and fungi 0.75 to 3.1 times as great in number in the rhizosphere as in the soil. The latter group of organisms was somewhat more abundant in the rhizosphere of oats and barley and the former in that of alfalfa. In general, an increased rhizosphere effect was observed with advancing age of the seedlings. Zukovskaya (136), working with potatoes, flax and clover, found that the microbial population of the rhizosphere was 100 times as great in the soil as from the root. Changes of this microflora coincided with development of the plant. She also claimed that each plant, grown in the same soil, enhanced the activity of a specific microflora of its own. Using flax, Berezova (5) concluded that different types of organisms were associated with plants at different stages of growth. Some were found only during certain stages of development, some during all stages and others were present only at maturity. This author is of the opinion that there are two distinct zones in the rhizosphere which support different microbial populations; one is the rhizosphere soil, containing aerobic bacteria, fungi and actinomycetes, and the other is the root surface with denitrifying organisms of the *Radiobacter* and *B. macerans* group. Borodulina (8) observed that the microflora of the seed coat develops and establishes itself in the plant rhizosphere; beneficial as well as harmful organisms can multiply in this zone, a fact which finds application in bacterial treatment of seed. Working with mangels in field plots, Katznelson (41) reported distinct effects of age of plant on bacteria, fungi, actinomycetes, algae and protozoa and on specific groups of organisms to be referred to later. For example, R: S ratios of bacteria on plants in untreated soil were 35, 45 and 113 at 80, 117 and 142 days; ratios for actinomycetes increased from 3 to 30 during this period, algae from 0.4 to 29.

Different rhizosphere effects may also be obtained with different varieties of one plant species. Obraztzova (78) found that the variety of tea-bush did not influence numbers of microbes but did affect their quantitative composition. Timonin (112) noted higher numbers of bacteria and, to a less extent, of fungi in the rhizosphere of varieties of flax and tobacco plants susceptible to soil-borne plant pathogens, though free from the disease, than in those of corresponding resistant varieties. Data by Lochhead and co-workers (65, 67*a*, 131) support this observation. It was suggested that these results reflected inherent differences in physiological function, making in the case of susceptible plants, conditions somewhat more favorable for general bacterial development. In this connection it is interesting to note the recent work of Eaton and Rigler (18) who showed that as the carbohydrate content of cotton roots increased, the total bacterial count on the root surfaces decreased, suggesting "that it is possible to produce, within a single variety of cotton, differences in bacterial numbers and root rot susceptibility somewhat analogous to those that exist between different varieties of flax and tobacco".

Environmental Factors. The influence of factors such as the type of soil, moisture content, reaction, treatment and temperature on the soil microflora has been thoroughly presented (120). Much less work has been done on the effects of these factors on the rhizosphere microflora. Thom and Humfeld (107) report considerable variations in absolute counts and R: S ratios of rye in three different soils. A Keyport clay loam gave ratios of 6 and 5 for fungi and bacteria as compared with 15 and 16 on roots of plants in a Collington fine sandy loam. In a study of corn growing in different soils varying from pH 4.5 to 8.1, bacteria were found to be more numerous under slightly acid or neutral conditions, fungi more abundant on roots in the most acid and most alkaline soil, whereas actinomycetes were not affected by soil differences. Adati's (1) work, referred to previously, showed that on the whole, R: S ratios were highest on loam (not sand, as claimed) with sand, clay and humus soils in order of decreasing effectiveness. This order held for the three major groups of organisms studied. The influence of soil type on the rhizosphere microflora has also been observed by other investigators (11), although it is not yet clear whether the effect is direct or indirect (*i.e.*, through influence on plant growth).

In general, soil treatment does not appear to affect numbers of organisms in the rhizosphere (10, 13, 30, 42, 78, 112). Liming was reported, however, to increase numbers by 25% (78). Manuring, though invariably inducing large increases in the microbial population of the soil, did not appear to exert a similar effect on the root population (10, 13). The effect of any treatment must also be evaluated in terms of its influence on the rate of growth of the plant (11, 41). For example, Katznelson (41) observed that numbers of bacteria, fungi, actinomycetes and protozoa reached a maximum in the rhizosphere of rapidly growing, vigorous mangel plants in manured soil at the height of the growing season. However, plants in unfertilized soil grew much more slowly, reaching near the end of the growing period a size comparable to that of the mangels in the manured soil at the peak of the growing season, and, as may be expected, exerted their full effect on the root microflora only when full grown. The importance of the R: S ratio in interpreting results is clearly demonstrated in this work. Counts of actinomycetes on mangel roots reached a peak at 117 days and decreased at 142 days; the R: S ratio was 23 and 30 for these two sampling periods, indicating an actual increase in the "rhizosphere effect".

It was also observed that at 142 days, counts of algae were considerably higher in the rhizosphere of plants in manured soil than in that of plants in unfertilized soil, yet the R : S ratios 15 and 29, respectively, indicated that the roots in the latter soil exerted a greater effect than those in the former.

Soil moisture content has been observed to influence numbers of organisms in the rhizosphere. On comparing soil and rhizosphere counts at different soil moisture contents, Clark (10) found that the R: S ratio did not vary appreciably over a range of 12 to 20% moisture (about 9), but at 24.5% the ratio dropped to 4.5. Under field conditions (11) it was somewhat more difficult to assess the influence of moisture, since ratios varied considerably. In studies on the influence of soil moisture content on numbers of microbes in the rhizosphere of flax, Timonin (112) observed that a lower moisture content of the soil (30% of the total moisture-holding capacity as compared with 60%) increased the density of the microbial population in the rhizosphere. This effect was especially striking with bacteria, next with fungi and least if at all with actinomycetes. Similar results were obtained (113) with "artificial rhizospheres" (collodion membranes), especially as related to the fungous population; there was little effect with actinomycetes. It has been suggested (10, 12) that these effects may be due more to increased or decreased adherence of soil particles to roots (in wet and dry soil, respectively) than to actual changes in numbers of organisms in response to altered environmental and nutritional conditions. However, more information is required on this point before it can be generally accepted.

Soil sterilization with various agents such as steam, formaldehyde and chloropicrin, is analogous to soil treatment in certain respects (120, 124). Since these agents are used to eliminate a disease factor from soil they should perhaps be left for consideration along with root rots in general. However, certain peculiar effects have been obtained which may be mentioned at this point. Katznelson and Richardson (42) found, for example, that steaming reduced bacterial and actinomycete numbers in soil very markedly for about three months, yet the numbers on the healthy roots of tomatoes were as high as on those in untreated soil, implying a protective and ameliorating influence of the plant on the soil microflora in the rhizosphere. Similar results were obtained with certain treatments, *e.g.*, dried blood in connection with control of strawberry root rot (43).

A number of investigations have been published concerning the influence of soil amendments on the quantitative and qualitative rhizosphere microflora in relation to root rot diseases of plants. Since the root rot condition introduces another factor contributory to the rhizosphere population, these findings will be considered in a separate section.

Carbon Dioxide Evolution. The abundance of organisms about plant roots presupposes intense activity in that region. This activity has been measured in terms of carbon dioxide evolution. It appears generally accepted that larger quantities of this gas are evolved from soils under plants than from unplanted soil. Considering the contribution of the plant in this connection, it is obvious that carbon dioxide arising from its growth may come from at least two sources, *viz.*, from the plant roots, as a product of root cell respiration, and from microbial activity about the roots (124) in decomposing organic excretions and "sloughed off" portions of the roots. Which of these sources supplies the greater amount of this gas depends on prevailing environmental conditions, stage of growth and nature of the plant. As much as 45% of this gas may be the product of microbial activity (124).

Slight effects were noted (95) in the early stages of growth and abundant formation of carbon dioxide when plants reached the stage of extensive vegetative development; following maturity, less gas was produced. Biennials were found to exert a prolonged effect, owing to their longer growth period. In general, then, the influence of plants on the formation of carbon dioxide was similar to the changes he reported of the bacterial populations of the rhizospheres of these plants (94). In further studies Starkey (96) reported greater amounts of carbon dioxide arising from soils obtained from regions of principal root development than from soils apart from the root; this was true with old (biennials) as well as with young (first year) plants. Again the correlation between intense carbon dioxide evolution and abundance of soil microbes was evident.

Stille (102) compared carbon dioxide evolution by mustard growing in sterile sand and sand inoculated with a soil suspension, and concluded that carbon dioxide production was due largely to microorganisms. Analysis of the data of Barker and Broyer (3) indicates that under aerobic conditions of growth the average carbon dioxide evolution by squash roots receiving manganese in sterile culture was 0.279 and in nonsterile culture 0.549 mgs., or slightly over 50% due to the roots. However, under anaerobic conditions the sterile system yielded 0.108 and the nonsterile system 0.289 mg., or 37% from the roots. It is clear from this limited information that no definite conclusions can be reached regarding the proportion of carbon dioxide produced by plant roots or the rhizosphere microflora. That the amount evolved is appreciable is quite apparent, and its influence on root feeding and microbial activity in the rhizosphere is of unquestioned importance (120, 124).

QUALITATIVE EFFECTS OF HIGHER PLANTS ON SOIL MICROORGANISMS

Perhaps even more significant than the quantitative effects of higher plants is their qualitative influence on the soil microflora. Studies on this phase of the rhizosphere problem are increasing in number, with emphasis almost exclusively on bacteria, but are still not abundant. In general, the procedures involved in these investigations have been along two broad lines: those in which numbers of specific groups or species are determined on selective media by plating or dilution methods, and those in which a non-selective medium is used and all colonies on a plate or a representative sector of a plate are picked and studied from the point of view of cultural, morphological or physiological behavior; by this means quantitative, as well as qualitative, data may be obtained, as well as information on the relative incidence of specific types of organisms. In addition, studies of prevailing types of bacteria adhering to buried slides have also yielded useful information. Little data exist, however, on the selective action of roots on the fungus flora of the soil and practically none concerning actinomycetes, protozoa, nematodes and algae with the exception of the parasitic forms within these groups which are involved in root diseases (6, 14, 21, 27, 91).

Since plants differ in chemical composition, physiology and nutrition, it is conceivable that they would attract specific groups or species of organisms which may be beneficial, harmful or without known effects. Decomposition of "sloughed off" root fragments may be carried out by specific organisms whose nature is governed by the chemical composition of these root parts, and excretion of specific and perhaps characteristic substances may favor the development of a rhizosphere microflora typical of a particular plant (20, 31, 32, 34, 51, 79*a*). Hiltner (28) held the view that this selective action is favorable for the plant, which is far from being the case, as the multitude of root rot diseases attests. Any factor contributing to a modification of growth and physiological behavior of the plant may, then, be expected to initiate changes in the root microflora. Such factors as age and nature of the plant, moisture content, reaction and type of the soil have been shown above to alter the "rhizosphere effect" on numbers of the broad groups of soil organisms. The following section deals with this "rhizosphere effect" on the incidence of specific groups or species of soil microorganisms. Bacteria have been studied most extensively in this connection and will be considered first.

Bacteria: Morphologic and Taxonomic Types. In this section it is intended to group all organisms without known or specific physiological function, such as "dye-tolerant bacteria", Gram-negative rods, spore-formers or specific types, as *Alcaligenes radiobacter.*[5] Organisms of the Radiobacter group and related forms usually appearing on the same selective medium employed (*Aerobacter* and *Rhizobium* types) have been widely mentioned as being stimulated in the root zone and especially in that of legumes (15, 24, 90, 93). For example, Starkey (94) found that organisms of the *B. radiobacter* group, producing mucoid colonies in nitrogen-free mannite agar, increased in the rhizosphere more than other groups studied; oats and rape showed the most pronounced effects, but potatoes exerted only slight influence. As plants reached maturity the rhizosphere effect diminished, though beans exerted their selective power up to the final stages of growth. Sweet clover also exerted a prolonged effect, as might be expected from a biennial. Even more striking results were obtained with this group by use of glycerol-nitrate agar; the influence of rape was particularly great; an R : S ratio of 86 was obtained at 44 days, decreasing to 9 at the final sampling period. Again with most plants the increase in rhizosphere effect was greatest at the height of the growing period followed by a decrease at the final stages of growth. In general, young plants exerted only a slight effect. Despite these large ratios, the absolute increases in most cases are much lower than with the other organisms studied, as this group of bacteria constitutes a small proportion of the total soil or rhizosphere population. Later Starkey (96), in a comparison of the effect of biennials and annuals at different distances from the roots on numbers of the *B. radiobacter* group, observed a marked selective action, especially with sweet clover; an R:S ratio at 356 days of about 254 was obtained which increased to 2,218 at 437 days. These large ratios were considered due to the inclusion of *Bacillus radicicola* in this group. Again the Radiobacter group increased proportionally more than any of the other groups studied.

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[5] Specific names of bacteria are based on Bergey's Manual (5th Ed.) except where derived from original articles in which case the name used is that employed by the author quoted.

The weight of evidence suggests that Gram-negative, non-spore-forming bacteria (Radiobacter types, fluorescent and "dye-tolerant" forms) multiply extensively in the rhizosphere of a wide variety of plants, whereas spore-forming bacteria do not (10, 11, 48, 50, 56, 57, 65, 78, 136). The latter can develop on roots (13, 102) and occur in greater abundance on those of certain plants (oats, radishes) than on others (barley, corn, beans, lettuce) (11). Certain spore-formers (*B. brevis, B. circulans, B. polymyxa*) were reported to comprise a more important fraction of the *Bacillus* population in the rhizosphere than in the soil; *B. polymyxa* appeared to be relatively more abundant on cereal roots and *B. megatherium* on cotton roots (11). Some plants actually repress these organisms in the rhizosphere (11, 65). Distribution of these bacteria on plant roots is also affected by the nature of the soil (11).

As may be expected, the nature and age of the plant affect numbers of Gram-negative, non-spore-forming rods also. "Dye-tolerant" bacteria were found to be more abundant on cotton than on wheat roots, and fluorescent bacteria highest on barley and radish roots (11). Lochhead (65) also observed that Gram-negative short rods were relatively more abundant, and coccoid forms less abundant in the rhizosphere of varieties of flax and tobacco susceptible to certain root diseases. Starc (92) reported that *B. fluorescens* developed rapidly on the root hairs of maize. Eaton and Rigler (18) noted the preponderance of blue green fluorescent bacteria on cotton roots with a high carbohydrate content. The early stages of growth appear to favor extensive development of these organisms (11, 56, 57, 136), although they are very abundant even in the later stages (50). Numbers of Gram-negative short rods in the rhizosphere are also affected by soil and climatic conditions (50).

Examination by the buried slide technique (30, 99, 100, 112) lends further support to the observed preponderance of Gram-negative non-spore-forming rods and the scarcity of spore formers in the rhizosphere.

Mycobacteria have been reported to occur in great abundance in the rhizosphere of certain plants (56, 57, 78). Thom *et al* (108) noted that the majority of organisms isolated from wheat roots in chicken-manured soil were Gram-positive corynebacteria, inhibited by crystal violet, reducing nitrate and generally requiring organic nitrogen for growth. Similar forms, though without the capacity to reduce nitrates, predominated on the roots of wheat on alfalfa-treated soil. Lochhead (65) reported that the *Corynebacterium* group and proactinomycetes were relatively less abundant in the rhizosphere of various plants than in soil apart from the root, but that *Mycoplana* was preferentially stimulated.

Valleau *et al.* (117, 118) found that certain bacterial pathogens of tobacco plants, such as *Bacterium tabacum,* causing wildfire, and *Bacterium angulatum,* causing angular leaf spot, occur in nature on the roots of pasture and crop plants. They remain indefinitely and apparently form colonies on the small rootlets of the plants. *Xanthomonas vesicatoria* also appears to grow and overwinter on wheat roots (16).

Bacteria: Physiological Groups. Perhaps even more important for an understanding of the interrelationships between plant roots and the soil microflora is an appreciation of the physiological activity and nutritional requirements of the organisms congregating at the root-soil interface. Carbon dioxide evolution from soil has frequently been used as an index of microbial activity and has been shown to be greater in soil adjacent to roots than in soil beyond the zone of root influence (74, 93, 100). This effect is related to the abundance of organisms in these soils and to availability of organic substances for decomposition, the rhizosphere soil containing more of both than soil apart from the root. Thom (106) pointed out that most of the organisms associated with the rhizosphere belong to species active in the decomposition of fresh organic matter and not to forms involved in the breakdown of humus residues in soil. Clark (11) noted that the *Pseudomonas* types of bacteria from soil differed from those associated with roots. Most of the soil isolates did not utilize sucrose or hydrolyze starch, whereas the majority of root isolates fermented the more complex sugars.

By means of plating on a non-selective medium and isolations Lochhead (65) found a more active bacterial flora in the rhizosphere than in the soil apart from the root. A higher percentage of forms growing well on nutrient agar, of liquefying types, and of those causing acid or alkaline reactions in dextrose were found in the rhizosphere. A noticeably greater percentage of motile bacteria and of chromogenic types was also noted on the root surfaces. Chromogenic forms occurred in greatest relative abundance in tobacco rhizospheres; other plants showed similar though not such striking differences. He observed a somewhat more active microflora in the rhizosphere of susceptible varieties of flax and tobacco.

Bacteria: Nutritional Groups. The method employed in studies of the qualitative nature of soil bacteria by Taylor and Lochhead (104) and Lochhead (65) was applied by West and Lochhead (131) to a study of the nutritional requirements of the organisms isolated from the non-selective (soil extract agar) medium as described earlier. Different cultural media, ranging from a simple inorganic basal medium to more complex substrates, containing amino acids, amino acids + growth factors, or yeast extract, were used. The roots of even young seedlings of flax and tobacco favored the development of bacteria dependent upon a supply of thiamine, biotin and amino acids for growth. Organisms growing in the basal medium were relatively more numerous in the flax rhizosphere than in the control soil; tobacco rhizospheres did not show this effect. Bacteria requiring or stimulated by amino acids were relatively more abundant in the rhizospheres of both plants than in the control soil and especially so in the rhizospheres of susceptible varieties (131). Similar observations were made with bacteria requiring, in addition, growth factors. Organisms requiring yeast extract were relatively more numerous in the control soil than in the plant rhizospheres in most cases. Greater differences were found to exist between the rhizosphere and the control soil where the latter was poorer than where it was richly supplied with organic matter whose decomposition would obviously yield amino nitrogen and growth factors for the soil bacteria. These results were confirmed (42) with greenhouse tomatoes and later (43) with strawberry plants. However, organisms requiring known growth factors did not appear to be preferentially stimulated in the rhizosphere (43, 67). Further studies (132) involved a slight modification of the media described above and the development of a means of assessing the equilibrium existing between these nutritional groups in soil—the Bacterial Balance Index (B.B.I.). This index was calculated by assigning a negative value to the percentage occurrence of Gram-negative bacteria growing abundantly in a simple inorganic medium, a positive value to those organisms requiring amino acids and growth factors, and adding the resulting figures. By this method it was shown that the rhizosphere of Premier strawberry seedlings had an index of +63 as compared with +35 in the control soil. This principle was later applied (29, 130) in a study of the rhizosphere of strawberry plants in relation to root rot and will be considered.

A more extensive differentiation of the nutritional groups of bacteria was proposed by Lochhead and Chase (66), which involved a determination of growth response in seven cultural media of increasing complexity. In an application of this procedure to a study of mangels, it was found (67) that bacteria with simple requirements and those responding to amino acids were preferentially stimulated in the rhizosphere. It was also reported that culture filtrates of bacteria growing in the simple inorganic medium provided greater stimulation to organisms requiring amino acids and were more antagonistic to bacteria requiring growth factors. Thus at least a partial explanation for the increased relative incidence in the rhizosphere of bacteria requiring amino acids may be found in associative action of the rhizosphere microflora, although other phenomena, such as root excretions and decomposition of root fragments, undoubtedly also play an important rôle in this connection. In addition to the relative increase in the rhizosphere of organisms with simple requirements and of those requiring amino acids for maximum growth, another characteristic effect is the relative suppression of bacteria dependent upon the more complex nutrients provided in soil extract (42, 43, 67, 126), findings confirmed by recent work (126) with greenhouse oats, wheat, flax, timothy, red clover and alfalfa.

Nitrogen-Fixing Bacteria—Rhizobia. Due to technical difficulties there are comparatively few actual counts of rhizobia in soil and in the rhizosphere of plants. However, it has been shown by a number of investigators (20) that growth of plants, especially legumes, results in greater numbers of rhizobia in the soil. It has been noted previously that organisms of the *A. radiobacter* group are abundant in the rhizospheres and particularly so in the rhizospheres of leguminous plants. Such counts undoubtedly include organisms such as *Rhizobium* (93), and it may not be unjust to infer that this group is also selectively stimulated. In subsequent work Starkey (95) suggests that the abundance of *B. radiobacter* and related forms about legume roots is due partially at least to the fact that *Bacillus radicicola* is included in the group and undoubtedly makes up a considerable portion of the bacteria developing from samples taken from legume roots. Rhizobia have been isolated from the root zones of a large number of plants in Russia (83), although there was no indication of their relative incidence in soil or in the rhizosphere. Plants of the family Pinaceae did not yield these organisms whereas roots of members of Polygonaceae supported largest numbers. Lochhead (64) noted distinctly larger numbers of *R. trifolii* in the rhizosphere of clover than in control soil; R: S ratios over 100 were obtained.

Koreniako (46) reported that root excretion of some plants (clover, peas) stimulate, while those of others (corn, flax) retard development of nodule bacteria in the rhizosphere. Nodule bacteria developed well in the rhizosphere of wheat, cotton and other non-leguminous plants. Later work (54, 55) reported that some *Pseudomonas* and *Achromobacter* types inhabiting the root zone influence strongly the development of nodule bacteria (*R. trifolii*) in the rhizosphere of clover. Certain species stimulated growth of the nodule bacteria as did *Azotobacter,* others inhibited and still others were without effect. The authors claimed, too, that less virulent strains of nodule bacteria are activated by mixing with the stimulating organisms. A further suggestion was made that nitrogen fixation occurs in clover cultures in the absence of nodules but that the presence and development of bacteria activating this process around and on the root is necessary. The claim that these activating bacteria produce biological catalyzers penetrating the plant and stimulating the process of nitrogen fixation by plant tissue requires further substantiation in the light of the widely accepted concept that both symbionts, the legume and the nodule bacteria, participate in this process (20, 133, 134).

Azotobacter. Despite the interest in these organisms from a theoretical and practical point of view, there is surprisingly little information of a quantitative nature concerning their natural occurrence in the rhizosphere of plants. Beijerinck as early as 1908 found larger numbers of *Azotobacter* in soil close to the roots of legumes than in soil from roots of non-legumes (93). Kostychev *et al.* (47) noted the accumulation of *Azotobacter* in the vicinity of tobacco roots. Starkey (94), however, could obtain no evidence of a selective action of roots of legumes or non-legumes on these nitrogen-fixing bacteria. Comparing samples from the rhizosphere and from soil apart from the root, Gräf (24) concluded that the most intensive nitrogen-fixation by *Azotobacter* and *B. amylobacter* occurred with the rhizosphere soil. It was strongest with samples from the root surface ("inner" rhizosphere) and also from the control soil in June, and greatest with the rhizosphere soil ("outer" rhizosphere) in July. A decline of intensity of fixation occurred with samples from all three zones in August. Soil from the rhizosphere of legumes exerted the most favorable influence; that from rape was also somewhat favorable, but samples from grains were without effect. Although these results imply greater numbers of nitrogen-fixers in the rhizosphere of some plants, they can also be interpreted on the basis of more active strains of *Azotobacter* rather than larger numbers in the rhizosphere. Others (115) isolated *A. chroococcum* from the rhizosphere of wheat, but in the absence of data on its incidence in control soil, no selective action by wheat roots can be deduced. The same objection applies to the much quoted work of Poschenrieder (80). Evidence was obtained merely on the presence of *Azotobacter* on roots of various cultivated and wild plants. The organism was almost always present on roots in the summer, though frequently absent in the fall, especially from plants which had been harvested. Comparing the "inner" and "outer" rhizospheres (washed roots and roots with adhering soil respectively) he found that the latter showed generally better development of *Azotobacter* than the former (various plants showed no *Azotobacter* in the "inner" rhizosphere). Extensive growth of fluorescent bacteria in cultures from the "inner" rhizosphere suggested that these organisms may have repressed *Azotobacter* in this zone. There is little to show that this organism is more abundant in the rhizosphere than in the control soil, or to support the statement that it is a "root bacterium". Rokitzkaya (83), in a study of the microflora of the root systems of plants belonging to 31 families, noted the presence of *Azotobacter* in all but three (Pinaceae, Betulaceae and Fagaceae). "Relative intensity" of development of *Azotobacter* was greatest with members of Polygonaceae, Rosaceae, Pomaceae and others. Krassilnikov (48) concluded that this organism was absent from the rhizosphere of wheat and developed only weakly in that of maize. By means of buried slides it was demonstrated (30, 99) that *Azotobacter*-like cells occur on roots of various plants. Lipman and Starkey (63) pointed out that there is considerable evidence that *Azotobacter* does not appear more commonly in the rhizosphere than elsewhere in the soil. However, Manzon (74) claims that fixation of free nitrogen is greater in the rhizosphere than in control soil, and others (88) report the accumulation of *Azotobacter* throughout the early stage of development of corn and buckwheat; at the flowering stage numbers were depressed and the organism gradually disappeared as the plant matured. In a study of growth of roots *in vitro* it was observed (53) that the presence of bacteria depressed growth, wheat roots being more sensitive to *Azotobacter* than pea roots; but that filtrates of *A. vinelandii* stimulated root growth. Sidorenko (89) also reported that oats, barley, sudan grass, sunflower and soybean stimulated growth of *Azotobacter* inoculated on seed, but that wheat depressed it; corn, soybean and alfalfa caused fluctuations according to the developmental stage of the plants, and sugar beet was inert in its effect on *Azotobacter* development. Clark (11) did not observe appreciable numbers of *Azotobacter* on the root surface of plants growing in soils themselves generally free of this organism, and Jensen and Swaby (36) could not demonstrate a marked prominence of *Azotobacter* in the rhizosphere of legumes. Even by means of heavy inoculation of roots of tomato seedlings, Clark (12) was unable to establish *Azotobacter* in the rhizosphere. However, Starc (92) reported increases in number of cells in sand and around roots of maize when tested in suitable media; yet by means of buried slides he could not detect them on the root hairs. More recently, Katznelson (41), using a dilution-plate method of counting

Azotobacter cells, demonstrated the presence of this organism in the rhizosphere of mangels and in soil apart from the root, but there was no evidence of a selective action by the roots. Further work (44) provided evidence of the presence of this organism in the rhizospheres of wheat, oats, timothy, flax, red clover and alfalfa, but only with flax was there an indication of a slight "rhizosphere effect". Timothy actually exerted a slightly depressing effect on the organism.

It would appear, therefore, that *Azotobacter* does not find the root zone of many, if not of most, plants a more congenial habitat than the soil proper, a fact which renders the procedure of seed treatment with this organism and the claims of resulting increases in crop yields somewhat suspicious. This question will be referred to later.

Anaerobic Nitrogen-Fixing Bacteria. Surprisingly little attention has been paid to the anaerobic nitrogen-fixing bacteria in the rhizosphere, especially in view of the fact that they are more abundant in soil and are more adapted to a wide variety of factors (moisture, temperature, reaction) than *Azotobacter* (120). Gräf (24) mentions *B. amylobacter* as occurring in the rhizosphere and in soil, as demonstrated by nitrogen fixation in mannite solutions, but there seems to be no evidence to show that this organism was actually isolated and identified. Bezssonoff and Truffaut considered *Clostridium pasteurianum* to be involved in supplying nitrogen to corn (38). Demidenko apparently did not observe a selective action on this organism by plant roots (63).

In a study of gas-producing anaerobes, very closely related to, if not identical with, *Clostridium pasteurianum* in the rhizosphere of mangels, Katznelson (41) observed a distinct rhizosphere effect throughout the growing season for roots in manured soil. In untreated soil this effect was observed considerably later in the growing season, at which time the R: S ratio was 200 as compared with 16 for plants in the treated soil. As many as 1.4 million bacteria per gram rhizosphere soil were found. In a later survey of various plants grown in the greenhouse (44), no marked stimulation of this group of organisms was observed except with flax which yielded an R: S ratio of 5.0 and 3.2 at two and four months, respectively. As with *Azotobacter*, counts of these gas-producing anaerobes appeared to be depressed by timothy. More work is required to elucidate the incidence and rôle of these organisms in the rhizosphere.

Ammonifying and Proteolytic Bacteria. Various spore-forming and non-spore-forming bacteria, such as *B. mycoides* and *Bact. fluorescens,* respectively, have been shown to play an active part in the breakdown of proteins and simpler nitrogenous compounds with liberation of ammonia (120). Certain of these bacteria are more effective together than when alone (123). As noted above, spore-formers, though occurring in the rhizosphere, are nevertheless comparatively scarce, but non-spore-forming organisms, of which fluorescent types are prominent, are the predominant forms on root surfaces. It is likely, then, that these are chiefly responsible for the evidence of large numbers of ammonifying bacteria on plant roots. For example, Gräf (24) noted that the decomposition of peptone and production of ammonia was strongest with samples from the root surface, increasing in intensity from May to July and falling sharply in August. Rape appeared to stimulate the process, grains showing only a slight effect and legumes least. Rokitzkaya (83) also demonstrated the presence of an active ammonifying flora in the root zone of 178 different plants. As might be expected, different plants exhibited different "intensities" of ammonification. Krassilnikov *et al.* (57) stated that the predominant forms in the rhizosphere consist of non-sporing rods with pronounced ammonifying capacity. Lochhead (65) observed that gelatin-liquefying types, and Timonin (114) that casein-hydrolyzing bacteria were more numerous in the rhizosphere of various crops than in control soils. Katznelson (41) demonstrated a striking stimulation of this group of bacteria on mangel roots from manured soil, an effect which increased with age of the plant and was more pronounced in treated than untreated soil. However, at the end of the growing season the R:S ratios for the roots in both soils were about the same (about 2,000). It is to be expected that in this zone of root influence, microbial activity would be intense, and it is logical to infer that such common soil processes as CO_2 evolution and ammonification would be particularly pronounced. Thom's (106) observation that most of the organisms associated with plant roots belong to species active in the decomposition of fresh organic matter is in line with the above findings.

Nitrifying Bacteria. Studies of the effect of plant growth on nitrification in soil are quite numerous, but investigation of numbers of nitrifying bacteria on the rhizosphere of plants practically nonexistent. Most investigators have compared samples taken from planted soil with those from unplanted soil, and the results (15, 71, 93) indicate that nitrates accumulate more rapidly under legumes than under non-legumes and that, in addition, plants exert an accelerating effect on nitrification in the early stage and depress it at more advanced stages of growth. Starkey (95) compared the accumulation of nitrate-nitrogen in fallow soil with soil obtained from the roots of various plants. In general, nitrates increased more rapidly in the latter soils and were more abundant in soil under biennials than annuals, the effect extending throughout the growing season. Nitrate formation was greater in soil supporting biennials at the last sampling than at any previous period with any of the other plants. Rape exerted the most pronounced effects throughout and potatoes least; however, no marked difference between the effects of legumes and non-legumes was observed. The influence of plants on nitrification was thought to be due to the addition of organic matter with a narrow carbon-nitrogen ratio to the soil resulting in liberation of ammonia on decomposition (120, 121, 124). It is, of course, possible that the development of plants causes an increase in numbers and activity of the nitrifying bacteria in the soil, but this could not be detected by the methods used.

Gräf (24) noted that nitrification was lowest at the root surface of plants but slightly higher in the rhizosphere soil than in the soil at a distance from the root. Rape showed no effect, however, and legumes were less favorable than grains. The rate of nitrification rose from May to June, dropped sharply in July and rose again in August. In a later study of this problem Starkey (96) again demonstrated that nitrification of soil nitrogen is greater in soil from regions of extensive root development than from soil supporting less root development, the results being especially pronounced with plants in more advanced stages of growth and more striking on the whole in clover than in table or mangel beet soil. From these data he considers it reasonable to suppose that the "soil constituents required by nitrifying bacteria are present in proportionately great abundance in soil close to the root". In addition, he states that "it may be concluded that either the nitrifying organisms in these soils are more efficient transformers of ammoniacal nitrogen or that these soils support a greater abundance of the bacteria". However, even though nitrate accumulation in soils from roots is greater than in fallow soil, the actual content of nitrate nitrogen of the soil decreases as the root is approached, owing to utilization of this nitrogen by the plant (97).

Truffaut and Vladykov (115) reported the isolation of *Nitrosomonas* from wheat rhizosphere, and Rokitzkaya (83) observed nitrification in the root zone of all plants tested. In an attempt to enumerate these organisms in soil and rhizosphere of mangels, Katznelson (41) demonstrated a slight rhizosphere effect of plants in manured soil especially at the early stage of growth.

Denitrifying and Nitrate-Reducing Bacteria. These organisms have been studied somewhat more extensively than the nitrifying bacteria. Gräf (24) noted that "nitrate assimilation" was strongest at the root surface, especially with legumes; rye was the best of the grains in this connection, whereas wheat and rape exerted the least effect. The presence of large numbers of denitrifying bacteria in the root zone of many plants has also been noted by other investigators (48, 56, 57, 58, 78, 83). Manuring appeared to stimulate these forms in the rhizosphere of mangels (41), although plants in unfertilized soil supported large numbers. Denitrifying bacteria were also reported to be more abundant in the root zone of an oat variety susceptible to manganese deficiency (114). Nitrate-reducing organisms were found in great abundance on wheat roots in manured but not in alfalfa-treated soils (108) and have been observed to be relatively more abundant in the rhizosphere of susceptible than of resistant strains of flax and tobacco (65).

Cellulose-Decomposing Bacteria. Cellulose-decomposing organisms, especially anaerobic types, have also received little attention with regard to their incidence in the rhizosphere. That organisms capable of decomposing cellulose are present on the root surface has been demonstrated by a number of workers (41, 78, 83, 115). Rokitzkaya (83) did not succeed in isolating *S. Hutchinsoni* (*S. cytophaga*) from the root zone of ten out of 31 families of plants. Anaerobic cellulose decomposers were isolated from all plant types studied, though, as with most of the data presented in this paper, there is no indication of a selective effect of the roots. Krassilnikov *et al.* (56) reported marked increases in cellulose-decomposing bacteria in the rhizosphere of wheat, maize, sunflower and soya. The same authors (57) noted a simultaneous increase of total numbers of organisms and of aerobic cellulose-decomposing bacteria in the rhizosphere of sunflower and soybean as the plants developed. An increase in numbers in the early part of the growing period was followed by a drop and then by another peak in the later part of the season. They concluded that these organisms take part in the decomposition of degenerated root fragments and that the products of this process are simultaneously used by other organisms. Aerobic cellulose-decomposing bacteria were found by Katznelson (41) to be abundant in the rhizosphere of mangels growing in manured soil but much more so in that of mangels growing in unfertilized soil. A rhizosphere effect with oats has also been reported (114); a slightly higher R: S ratio in the rhizosphere of an oat variety susceptible to manganese deficiency disease was noted.

Manganese-Oxidizing Bacteria. This group of organisms has been studied chiefly in connection with manganese deficiency disease of plants, particularly "grey speck" of oats. Gerretsen (22) suggested that this disease might be related to the presence of manganese-precipitating bacteria in the soil, pointing out that these organisms might be especially active in the neighborhood of the root where easily decomposable organic matter is available.

Timonin (114) observed that a susceptible variety of oats harbored in its rhizosphere a denser population of manganese-oxidizing bacteria than did a resistant variety grown under identical conditions. Partial sterilization of the soil with various substances (chloropicrin, formaldehyde, *etc.*) reduced markedly the number of these bacteria in the rhizosphere (and soil) and resulted in great improvement of the plants; the disease symptoms were also greatly reduced.

Anaerobes. It is surprising that this group of organisms has received so little attention from investigators of the rhizosphere. Yet the amounts of carbon dioxide evolved by roots and by their rhizosphere microflora might be expected to create conditions favorable for development of facultative and possibly even obligate anaerobes. Velich (119) found an organism called *Clostridium gelatinosum* Laxa in greater abundance in soils close to the roots and on the root epidermis of beets, mangels, barley, rye, oats and wheat. Similar observations were made by Maassen and Grüber (69). Rokitzkaya (83) demonstrated the presence of *Clostridium* in the root zone of 31 families of plants. The "intensity" of its growth was greatest with plants belonging to Chenopodiaceae, Solanaceae and Valerianaceae, and least with those of Pinaceae, Betulaceae and Fagaceae, the same families which yielded no *Azotobacter.* Katznelson (41) observed a striking increase in numbers of anaerobic forms on the root surface of mangels from both manured and untreated soils and especially from roots in the latter. This work was later extended (44) to include wheat, oats, flax, timothy, red clover and alfalfa growing in the greenhouse. Again, the roots supported much greater numbers of these organisms than the soil at a distance from them. R : S ratios were observed to increase with age of plants except for timothy which showed a lower ratio as it matured. Little else can be stated at present concerning this group of organisms except that it warrants further investigation.

Fungi and Other Micro-Organisms. Although many quantitative studies of fungi in plant rhizospheres have been reported, few data of a qualitative nature are available. Thom and Humfeld (107) reported that corn roots in acid soils yielded predominantely *Trichoderma* colonies, whereas roots from alkaline soil seemed to be associated chiefly with penicillia of the biverticillate series (*P. luteum, etc.*). Timonin (111) concluded that there is no significant difference in the fungal population of the rhizosphere of seedling plants (wheat, oats, alfalfa and clover) or between the rhizosphere and the soil distant from the root. However, in later work with flax he (113) reported that of 19 genera isolated nine were obtained from both rhizosphere and control soil and ten from the rhizosphere only. Certain genera—*Alternaria, Aspergillus, Cephalosporium, Fusarium, Helminthosporium* and *Verticillium*—were numerically more abundant in the rhizosphere of a variety of flax susceptible to root rot, whereas others, such as *Mucor, Cladosporium, Hymenula, Penicillium, Scolecobasidium* and *Trichoderma,* were more abundant in the rhizosphere of a resistant variety.

Katznelson and Richardson (42), in a study of the qualitative differences in fungi between the rhizosphere of tomatoes and the soil, obtained some evidence of a selective action by roots, especially among the "unidentified" fraction of the fungus population. Results varied with age of plant and soil treatment. In later work with strawberries these workers (43) also obtained evidence of qualitative differences among the fungi on the root surface as compared with those in the control soil. The untreated soil series yielded dark green *Penicillium, Aspergillus* and *Fusarium* types which were absent from the roots growing in it, which, however, yielded *Cladosporium, Chaetomium* and an unidentified non-sporulating fungus. Soil treatment affected this distribution, as was also found by West and Hildebrand (130) whose work will be discussed later. The rhizospheres of plants in soil treated with acetic acid supported a red-green form of *Penicillium* (26%), *Verticillium* (28%) and *Cladosporium* (4%), whereas the soils yielded none of these but contained *Aspergillus* (6%), *Trichoderma* (12%), *Oothecium* (16%), an unidentified fungus (24%) and other forms, such as *Mucor* and *Fusarium.* These results indicate a selective action of roots on soil fungi, but again more work is needed along this line.

The remaining groups of soil microorganisms, such as protozoa and algae, have scarcely been studied from a qualitative point of view. Attempts to determine whether roots of field mangels, or of wheat, timothy, oats, flax, red clover and alfalfa exerted a selective action on protozoa (41, 44) were not successful. Ciliates, flagellates and ameboid forms were all observed in both soils and rhizospheres. Blue green algae were noted (41) to be somewhat more abundant in the rhizosphere of mangels than in soil apart from the plant.

OTHER MICROBIOLOGICAL PHENOMENA ASSOCIATED WITH PLANT ROOTS

The great majority of the organisms discussed in the preceding pages develop readily in the soil or at the soil-root interface under suitable conditions. Whatever influence they may have on the root is exerted from without. Some of the organisms in the rhizosphere lead a passive existence, exerting little if any detectable effects. Others penetrate the root and establish a more intimate relationship with it which may be beneficial, harmless or injurious to the plant as a whole. Among the organisms concerned in favorable associations with plant roots, the nodule bacteria and mycorrhizal fungi are the most important. The invasion of plants, especially non-legumes, by bacteria resulting in the so-called "bacteriorrhiza" (20, 28, 79*a*, 133) may involve true symbiosis or may result in an entirely passive association (93). Of the forms concerned in harmful effects, fungi causing root diseases are the most numerous and economically important. In general, "the extent of penetration and degree of the effects vary with the soil conditions which determine the vigor and degree of resistance of the plants" (93). The nature of the organisms concerned (virulence, physiological state) also exerts a profound influence on the extent of invasion and severity of the effect (21, 27). Since all these associations involve highly specialized organisms with specific characteristics it may perhaps be argued that they should not be considered representative of the general microbial population of the soil or rhizosphere (21, 96). Nevertheless, they are involved in the microbiology of root surfaces and consequently deserve mention.

The symbiotic relationship between the nodule bacteria or rhizobia with leguminous plants, resulting in the fixation of atmospheric nitrogen, is the most thoroughly understood and most exhaustively studied of the beneficial associations between plants and soil microorganisms. Attracted by some specific substance or substances excreted by the young legume root, the nodule bacteria, after inducing a deformation or curling of a root hair, penetrate the root, multiply in the cells of the host and stimulate production of the nodule. The various factors influencing these steps are thoroughly summarized elsewhere (20, 133). One of the most important of these is the presence of combined nitrogen, such as nitrate, in the soil. This nitrogen apparently does not so much affect the organism as it does the host plant; it does not prevent entry of the bacteria but does inhibit nodule formation and depresses nitrogen fixation. These effects are believed to involve the carbohydrate-nitrogen balance in the plant, a subject which has been reviewed exhaustively (77, 133). Not all strains of rhizobia are capable of penetrating the root, nor are all effective in stimulating nodule production, or in nitrogen fixation, once the nodule is formed; some strains are even parasitic (110). On the other hand, some legumes do not form nodules (20).

Nodules in roots of plants other than legumes, such as alder, silver-berry and red root, have also been reported (20, 120, 124). In the majority of cases the organisms concerned have not been clearly defined nor their rôle in this association (20, 120).

Invasion of many plants by various bacteria is not uncommon (20, 28, 31, *79a*, 93, 96, 133). However, here again it has not been generally established to what extent these "bacteriorrhizal" formations are passive or beneficial. There is some question as to whether or not these are the true rhizosphere organisms as compared with other forms existing on the surface of the root or in the film of soil surrounding it. These may be the forms deriving some or slight benefit from the host, possibly contributing materially to it and detectable only by microscopic examination of roots or by culturing root fragments after washing the roots. Some investigators (31–35) feel that in these "bacteriorrhiza" we are dealing with an association similar to that of mycorrhizae.

The penetration of woody and herbaceous plants by certain fungi, resulting in "mycorrhizal" formations, is generally believed to be beneficial and in some instances essential to the host (9, 26, 120). Under unfavorable conditions, however, these fungi may even become parasitic (21, 72, 120). In some cases the fungus does not actually penetrate the root cells but forms a mass of mycelium around it, entering the root tissue chiefly between the cells; this type of mycorrhiza, very common among forest trees, especially evergreens, is called "ectotrophic". Other fungi penetrate root cells and may develop extensively therein; these are called "endotrophic" and are common in orchids and heather. According to various authors, mycorrhizal fungi supply their host with nitrogen, carbohydrates, water, phosphorus and other nutrients, auxins and enzymes and B vitamins (73, 75). However, "the nature and extent of the benefit to the host depends on various environmental conditions and the physiological processes involved in specific associations" (120). The benefit derived by evergreens from mycorrhizal fungi is of considerable practical importance in reforestation projects (21, 25, 81, 82).

A great variety of organisms occurring in the soil, such as nematodes, bacteria and fungi, can invade root tissue and cause serious damage to or even kill the plant (27). Attempts at control include use of inorganic and organic soil amendments (17, 21, 63, 120); partial sterilization with steam and other agents such as chloropicrin, formaldehyde (39, 76), and biological methods involving use of specific antagonists for particular pathogens (122, 127, 128); use of antibiotics (23, 122); or use of soil amendments designed to stimulate development of saprophytic organisms, thereby suppressing or eliminating the pathogen (85, 122).

In the rhizosphere the phenomena of association, antagonism, and competition for oxygen and food are perhaps even more intense than in the soil proper, owing to the denser population at the root surface and its greater physiological activity. Here the influence of root physiology and excretions may play an important rôle directly by stimulating or repelling the pathogen or indirectly by affecting the rhizosphere microflora (85). It has been pointed out earlier (65, 112) that plants susceptible to certain root rots induce a greater rhizosphere effect than corresponding resistant varieties, suggesting "that resistance to a certain disease may be linked up with a selective action of root excretions upon the saprophytic soil microflora thus favoring types which may be more and in other cases less antagonistic (directly or indirectly) towards pathogenic organisms" (65). In this connection it has been demonstrated (113) that a wilt-resistant variety of flax excreted hydrocyanic acid into the surrounding medium, thus exercising a selective power upon the fungus flora of the rhizosphere. The cyanide depressed pathogenic fungi, such as *Fusarium* and *Helminthosporium,* but appeared to favor *Trichoderma viride,* an organism mentioned frequently for its ability to suppress development of other fungi (122, 127, 128). On the other hand, by-products of growth of a susceptible variety stimulated growth of the two pathogens. Eaton and Rigler (18) showed that cotton plants with low carbohydrate concentration were most severely attacked by *Phymatotrichum omnivorum,* had largest number of bacteria and lowest number of blue-green fluorescent bacteria in their rhizospheres, and suggested that the resistance of the cotton to root rot reflected antibiotic protection at the high carbohydrate level. In further work they demonstrated that the immunity of maize plants to *Phymatotrichum* root rot is attributable to protection afforded by its root-surface microflora but that resistance of seedling cotton plants to *P. omnivorum* may be chemical.

Since soil treatment exerts marked effects on the soil microflora (saprophytic and parasitic), the question naturally arises as to whether similar effects may be expected in the rhizosphere. Clark (10) and Clark and Thom (13) claim that organic amendments applied to soil have no apparent effect on the root surface microflora. It was later (103) suggested that "once the root parasite reaches the susceptible plant, factors of nutrition, host resistance or parasite virulence are probably more important than microbial antagonisms". Katznelson (41) obtained definite evidence of stimulation of certain groups of organisms in the rhizosphere of mangels growing in manured soil, although it was not entirely clear whether this effect was due directly to the treatment or attributable indirectly to its influence on rate of growth and vigor of the plant. The effect of soil treatment on the qualitative nature of the soil microflora on plant roots has been brought out by Hildebrand and West (29) working with strawberries. Steam sterilization of soil or the repeated incorporation of soybean into root rot soil completely controlled the disease, whereas use of red clover did not. By means of the qualitative-nutritional approach discussed earlier they showed that these treatments had altered the bacterial equilibrium in both soil and rhizosphere so that untreated root rot soil gave bacterial balance index values of −46 and +10 for rhizosphere soil and soil apart from the root, respectively, as compared with +44 and +35 for steam-treated soil. General agreement was noted between this index and severity of the disease. No such correlation, however, was observed in relation to manganese deficiency disease of oats (114). Hildebrand and West suggested that the organisms responsible for lowering the bacterial balance index may actually be involved in production of the disease symptoms. However, Katznelson and Richardson (43) pointed out that it is possible that these organisms are secondary rather than primary invaders, increasing in abundance on the root following infection by other organisms, such as nematodes or fungi, or as a result of treatment and thus giving a low bacterial balance index. In this connection it should be pointed out in general that qualitative or quantitative rhizosphere studies of plants with diseased roots are complicated by the fact that once the root has been penetrated by the parasite, other organisms usually gain entry, decompose the root tissue and multiply, thus giving an abnormal and possibly a false picture of the rhizosphere microflora (10, 107). West and Hildebrand (130) showed in addition that the decomposition of soybean or glucose in root rot soil, or the addition of acetic acid, resulted again in a favorable alteration of the bacterial equilibrium (*i.e.*, higher bacterial balance index) and permitted growth of strawberry plants with well developed healthy root systems. These treatments also modified the fungus flora on the roots of strawberry seedlings grown in the treated soil so that potentially pathogenic forms (*Fusarium*, *Rhizoctonia*, *Cylindrocarpon*) were replaced by presumably harmless ones (*Penicillium, Mucor,* actinomycetes and yeasts). Katznelson and Richardson (43) in general obtained very similar results, particularly with fungi. The results with bacterial groups in the rhizosphere were not very consistent, due perhaps to the fact that the treatments used were not applied as frequently as they were by Hildebrand and West (29). Of particular interest was the fact that dried blood when added to root rot soil also induced healthy root formation. It was also noted that fungi with pathogenic propensities were more susceptible to acetic acid than normal saprophytic forms and that some of the former produced substances in culture which induced rapid wilting and drying up of strawberry seedlings (43). This type of work offers a promising line of approach to an understanding of the many problems involved in root rot diseases of plants, especially those of complex or unknown etiology. In this connection consideration should also be given to the influence of soil treatment on the physiology, vigor and resistance of the plant itself (21, 63, 125, 135) as well as on the pathogen. According to Walker (125), "there is reason to believe that studies of host nutrition in relation to disease development may yield information on the basis of which modifications in soil practices may often be used to reduce the acuteness of disease losses".

Seed Inoculation (*Bacterization*). Although soil and seed inoculation are artificial means of enriching soil, especially soil in the root zone, with specific organisms capable of establishing intimate associations with some plants, no review of the rhizosphere would be complete without some mention of this practice. The benefit derived by legumes from transfer of soil containing root nodule bacteria or by treatment of seed with pure cultures has been long recognized, and the latter procedure has become accepted agricultural practice. Inoculation of nursery beds with soil containing mycorrhizal fungi has also been shown to be an important step in raising planting stock in new nurseries and as an aid to the establishment of exotic species of forest trees (81, 82, 120). Bacterial treatment of seeds of non-legumes (bacterization), especially with *Azotobacter,* has often been attempted, but the results have been disappointing. However, there has been a recent revival of interest in this field, especially in Russia, where bacterization is being applied on a large scale and is considered of great agricultural and scientific importance. Claims of increases in yield of a large variety of crops, such as potatoes, sugar beet, oats, barley, flax, cabbage, wheat and tomatoes, have been made (38, 86, 87, 137); wheat yields, for example, increased from 123 to 484 pounds per acre; cabbage, 5,368 to 15,480; tomatoes, 2,904 to 11,600; and potatoes, 1,672 to 7,920. However, not all workers in Russia have reported such favorable results (38). Turchin (116) obtained no significant benefit from "Azotogen" or another similar preparation applied to a large number of crops in pot and field experiments. Nitrogen fertilizers gave large responses under similar conditions. Jensen (37) has recently assembled and critically reviewed the pertinent Russian literature.

Use of *Azotobacter* is based upon the premise that organic root excretions of plants are able to support sufficient growth and fixation of nitrogen by this organism to satisfy the nitrogen requirements of the plants. This presupposes that *Azotobacter* is an important rhizosphere organism, a theory not supported by the present weight of evidence.

Starc (92) inoculated sand growing maize with *Azotobacter* but obtained no increase in yield of plants; he suggested that these nitrogen-fixing bacteria use products of living and dead root portions only through the activity of other organisms. In addition, he observed that other bacteria and molds, especially *B. fluorescens,* rapidly developed on the root hairs so that *Azotobacter* was suppressed in the "inner" rhizosphere. Most recently Clark (12), in attempting to establish *Azotobacter* on roots of tomato seedlings by dipping them into heavy suspensions of *Azotobacter* and adding cultures to the roots on transferring from flats to soil in pots, observed that the organisms did not persist on the root but diminished in numbers rapidly over a period of weeks. In addition, Jensen (37) states that "no evidence has been produced that the quantities of organic matter secreted by the roots are upon the whole sufficient for nitrogen fixation of any significance in comparison with the nitrogen requirements of the plant". He inclines somewhat more to the suggestion of Isakova, Berezova, Krassilnikov and others that the effect of bacterization is due to stimulation of plants by growth factors elaborated by *Azotobacter* and other bacteria used for seed treatment, such as *Pseudomonas* spp., root nodule bacteria, tyrosin-decomposing bacteria and crude bacterial mixtures of rhizosphere organisms from different plants. Synthesis of various growth factors by *Azotobacter* and other organisms has been demonstrated by many investigators (40, 45, 109). However, Jensen (37) contends that "stimulation of intact plants by extraneously supplied growth-compounds seems to be the exception rather than the rule" and that "no proof has been given that bacterization results in a permanent modification of the rhizospheral microflora of such a character that organisms with particular aptitude for synthesis of growth-compounds become more prominent than otherwise". In this connection it should be pointed out, as already indicated, that organisms of the *Pseudomonas* type have been found in great abundance in the rhizosphere and that these bacteria are richest in growth substances, followed by *Azotobacter* and *Achromobacter* types with spore-formers and mycobacteria-producing none (52). More recent work (7, 101) casts some doubt on the importance of an external source of vitamins for higher plants, although the possibility that they may benefit at least to some extent from specific growth factors in soil, and particularly from those synthesized by the multitude of organisms in direct contact with the root (63, 101, 133) can not be entirely dismissed. Perhaps of greater significance are the claim of Isakova (35) that the rate and percentage of germination of seed can be increased by bacterization and the claim of a number of Russian workers that bacterial treatment of seed may exert a protective effect against infection of seedlings by parasitic fungi, such as *Fusarium* and *Colletotrichum* spp. (37). These investigators found that *A. chroococcum, P. fluorescens* and certain mycolytic fungi were active in this respect. Although it is difficult to consider *A. chroococcum* in the light of an antagonist, the use of specific antagonists for controlling soil borne parasites has been attempted by many investigators with varying success (122, 127). In regard to the influence of bacterization on seeds Jensen (37) agrees that favorable effects may be obtained, at least with seed of low germination capacity, as a result of the presence of bacteria capable of synthesizing growth-accessory compounds. However, Bawden (4) observed no stimulation of barley germination by plant hormones such as indole-3-acetic acid, α-naphthalene acetic acid and related compounds. Von Euler and Perje (19) also failed to note any effect of para-aminobenzoic acid, nicotinic acid, nicotinamide or adenosinephosphoric acid on seed germination; in fact, the first of these compounds was very inhibitory at certain concentrations. Recent reports from Russia and eastern Europe indicate very wide use of bacterial fertilizers, even to the extent of two and a half million hectares in the U.S.S.R. Emphasis appears to have been placed on selection of strains of *Azotobacter* specifically adapted for symbiosis with an individual species of plant (2). Again the effect of inoculation is claimed to be threefold: delivery of growth accessory substances, increase in the nitrogen content of the plant, and marked increase in the harvest yield of the crop. Apparently in no case was it possible for the individuals returning with these reports to assess the value of this work, and consequently much careful and critical research is required to substantiate the claims made.

DISCUSSION

Although adequate explanations for the congregation of organisms on root surfaces and for the preferential stimulation of specific types or groups are, in most cases, not available, these phenomena are unquestionably intimately related to the supply of inorganic and organic nutrients at this root-soil interface (94). Plant excretion of inorganic substances (70, 79, 93), accessory growth factors (68, 129, 133), amino nitrogen (68, 133, 134) and a variety of other substances (68, 93, 96, 99) have been generally accepted. Nor should the food materials supplied by "sloughed-off" root caps, root hairs, cortical and epidermal cells be overlooked, since they are rapidly attacked by soil microorganisms and supply considerable quantities of organic and inorganic nutrients (106). In fact, one of the most difficult technical problems in the study of the influence of higher plants on soil microorganisms concerns the distinction between actual excretory products of the root and the products of decomposition or autolysis of "sloughed-off" root fragments. It is not unlikely that some instances of observed "excretion" by plants should be attributed merely to breakdown of portions of the root.

As a result of this increased supply of nutrients in the rhizosphere the organisms normally present in soil, including fungi, bacteria and actinomycetes, and especially those capable of decomposing fresh organic matter (106, 120, 124) or of utilizing inorganic and simple organic nutrients directly, begin to multiply. Accompanying this increased biological activity are various microbial interactions such as symbiosis, mutualism, stimulation and inhibition with their resultant effect on the microbiological equilibrium, as well as on the plants (21, 63, 67, 122). It is quite conceivable that the observed increases or decreases in numbers of specific groups of organisms in the rhizosphere are due as much to associative or antagonistic effects as to the influence of the root itself (67). Excretion of inorganic elements such as potassium or phosphorus by plants may favor the development of bacteria with simple nutritional requirements (42, 43, 126, 131) in the rhizosphere, and liberation of amino acids may stimulate those requiring these compounds. Breakdown of root parts may also yield such substances and result in relative increases of these groups. On the other hand, the decreased relative incidence in the rhizosphere of bacteria requiring complex unidentified substances in soil extract (42, 43, 67, 126) may be explicable on the assumption that these organisms are more closely associated with breakdown of humus residues (121) than of the fresh organic material present at the root surface, or on the hypothesis that they are relatively depressed by the rapid development of the microorganisms (bacteria, fungi and actinomycetes) capable of utilizing the latter substances.

Bacteria oxidizing ammonia-nitrogen might be expected to be stimulated in the root zone as a result of the liberation of ammonia, due to the extensive development and activity of ammonifying organisms in the rhizosphere. Furthermore, as a result of the presence of both nitrate-nitrogen and appreciable quantities of carbon dioxide, denitrifying bacteria, as well as other facultative or even obligate anaerobes, might be stimulated. Accompanying the decomposition of readily available organic materials in the rhizosphere or subsequent to it, cellulosic substances are attacked, resulting in large numbers of cellulose-decomposing bacteria (and likely of fungi as well). Anaerobic bacteria can develop at the expense of the products of excretion, decomposition or cellular autolysis. With such extensive bacterial proliferation protozoa may be expected to find this zone a rich storehouse, provided antagonistic phenomena do not intervene to suppress them. The failure of such forms as *Azotobacter* and aerobic spore-forming bacteria to thrive in this environment may be ascribed to competition for food, susceptibility to antibiotics (which appear to be on the whole more toxic to Gram-positive bacteria) or to the fact that these organisms are less adapted to the facultative conditions obtaining in this zone. There are suggestions that different plant species or even varieties may favor the development of a specific rhizosphere microflora, but much more work is required before this can be regarded as established. It is of course recognized that the rhizosphere of certain groups of plants is characterized by the presence of specific organisms such as rhizobia and mycorrhizal fungi. This phenomenon is, however, distinct from the preferential stimulation by plant roots of soil microorganisms not regarded as symbionts or pathogens.

It is evident that the rhizosphere is a unique zone, exerting on many soil organisms a powerful stimulation which varies with the type, variety, age and vigor of the plant and the type, treatment and moisture content of the soil in which it grows. Environmental factors may exert their influence directly on the soil and the rhizosphere organisms or indirectly by stimulating or retarding plant development. Increased knowledge of the specific effects involved in this zone of interaction between roots and microorganisms may provide a basis for a better understanding of various phenomena related to plant feeding and growth, cropping systems and root disease control. The fields of investigation which such problems open are of great interest both academically and practically.

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ADDENDUM

Since the submission of this paper for publication several articles have appeared which have a direct bearing on the subject matter included in this review and which, therefore, should be noted.

ALLISON, F. E. *Azotobacter* inoculation of crops: I. Historical. Soil Sci. **64**: 413–429. 1947.
———— *et al.* *Azotobacter* inoculation of crops: II. Effect on crops under greenhouse conditions. Soil Sci. **64**: 489–497. 1947.
CLARK, F. E. *Azotobacter* inoculation of crops: III. Recovery of *Azotobacter* from the rhizosphere. Soil Sci. **65**: 193-202. 1948.

Allison *et al* observed no significant effect of inoculation on growth or nitrogen content of barley, Sudan grass, kale, rape, rye or Swiss chard. There was strong evidence against the theory, held by several Soviet scientists, that *Azotobacter* when living in the rhizosphere of plants benefits higher plants by fixing considerable quantities of nitrogen. Furthermore, no evidence of increased yields resulting from the production by *Azotobacter* of growth-regulating or stimulating substances was observed. Clark obtained no evidence that *Azotobacter* responds favorably to the presence of plant roots.

SCHMIDT, E. L. Mycorrhizae and their relation to forest soils. Soil Sci. **64**: 459–468. 1947.

This is a general discussion dealing with the various aspects of the mycorrhiza problem.

VEGETABLE SEED TREATMENT

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INTRODUCTION

The treatment of agricultural seeds as a control of disease began as early as the seventeenth century. Apparently the first chemical used was sodium chloride. Arsenic and mercuric chloride were suggested in 1756 (5), but neither was adopted. At that time attention was being directed almost entirely to wheat and to control of bunt (*Tilletia tritici* (Bjerk.) Wint.). Lime water was used with some success in 1755 (67). Copper sulfate solution as a seed drench was reported in 1761 (63), and its use increased gradually for a century. Prevost (61) in 1807 demonstrated that copper prevents germination of spores of the bunt fungus. Addition of lime to copper sulfate to reduce toxicity of the latter to seed had come into practice by 1862 (12). By 1889 wheat seed treatment with copper sulfate had become quite common in England (58).

While control of bunt was fairly satisfactory it became recognized that loose smut (*Ustilago tritici* (Pers.) Rostr.) is not affected by the copper-sulfate treatment (58). Suspecting that the latter fungus was more deep-seated in the seed than the bunt organism, Jensen (42), in Denmark, devised the hot water treatment, described in 1888, which is still used for loose smut of barley (*Ustilago nuda* (Jens.) K. & S.) and loose smut of wheat. The European work was confirmed and extended in United States (8, 44, 45, 46, 47). In 1895 Guether (33) in Germany was the first to report the treatment of grain seed with formaldehyde solution. Bolley (11) in 1897 reported favorable results with the latter chemical in United States, particularly on oats for the control of loose smut (*Ustilago avenae* (Pers.) Jen.) and covered smut (*Ustilago levis* (K. & S.) Magn.). By the turn of the twentieth century, methods had been worked out for the control of several seed-borne pathogens of cereals. Little attention had been given to methods of treatment for vegetable seeds.

TYPES OF SEED TREATMENT

At present there are recognized three distinct types of seed treatment. They are designated herein as seed disinfestation, seed disinfection and seed protection. The objectives of each will be described briefly.

Seed Disinfestation. Seeds are commonly contaminated on the surface by spores or other forms of pathogenic organisms, without being penetrated or infected. A majority of the grain-smut organisms bear such a relation to the seeds of their respective hosts. Chemical dips or soaks are eminently successful as seed disinfestants. The early success of copper sulfate against bunt was as a disinfestant. Numerous pathogens which contaminate vegetable seeds are controlled by chemical means.

Seed Disinfection. The failure of copper sulfate to control smut of wheat not only led to exploration for a better treatment but it also gave a clue to the life cycle of the loose-smut organism. When Jensen reasoned that heat might be more penetrating than chemical ions and less phytotoxic he suggested for the first time the principle of seed disinfection. As used here, then, disinfection refers to ridding the seed of a pathogen which has penetrated, infected and thus has become established within the seed. Hot water is still the standard treatment for loose smut of wheat and loose smut of barley, and it has been adapted to use with a number of vegetable seeds. Some chemicals are seed disinfectants as well as disinfestants.

Seed Protection. Seed protection refers to the treatment of seed, usually with chemicals, neither to kill organisms on the surface nor to kill organisms which have penetrated beneath the surface. Rather, it is designed to protect the seed and the young seedlings from organisms in the soil which might otherwise cause decay of the seed before germination or parasitize the seedling at or immediately following germination. The first successful use of a seed protectant was by Thaxter (66) in 1890 in connection with onion smut (*Urocystis cepulae* Frost). He had determined that the smut spores infest the soil and that the seedling is susceptible for a short time after germination of the seed. He reasoned that a chemical in close proximity to the seed in the soil might protect the young seedling during this short susceptible period. After trying several materials he found a mixture of sulfur and lime to be the most effective. This treatment was used for a time and was later supplanted by the formaldehyde-drip treatment reported in

1900 (62). Chemicals used with this objective are known as "seed protectants".

MAJOR TRENDS IN VEGETABLE SEED TREATMENT

As already indicated, little attention was paid to vegetable seed treatment before 1900. The use of sulfur and lime and later of formaldehyde as protectants applied to the soil with the seed to control onion smut has been mentioned. In the last decade of the nineteenth century the treatment of potato tubers with mercuric chloride and with formaldehyde came into use (4, 9, 10). The recognition of seed-borne pathogens of vegetables gradually directed attention to seed treatment.

In 1904 Harding, Stewart and Prucha (35) concluded that cabbage seed is commonly contaminated with the black-rot organism (*Xanthomonas campestris* (Pam.) Dowson). They suggested soaking seed in 1–1,000 mercuric chloride solution for 15 minutes or in formalin (40% formaldehyde), one pound to 30 gallons. The appearance of black leg of cabbage (*Phoma lingam* (Fr.) Desm.) in United States was recorded in 1910 (52). Henderson (36) demonstrated that the causal organism is seed-borne. While seed treatment with mercuric chloride or formaldehyde served to control the two diseases, growers and investigators became aware that epidemics might arise in spite of treatment. It was shown later that *Phoma lingam* (69) and *Xanthomonas campestris* (19) are both borne to some extent within the seed. Mercuric chloride and formaldehyde serve well as seed disinfestants but not as seed disinfectants. Another fact peculiarly patent to vegetable growing, when plants are often started in crowded plant beds, is that a very rare escape of the pathogen from the effect of seed treatment is often sufficient to start an epidemic in the seed bed when favorable environment prevails. This was shown to be a very important phase in the disease cycle of cabbage black leg and black rot, and it applies in much the same way to diseases of many other vegetables. The need for improved seed treatment of cabbage led to exploration of other methods. Dry heat was not practicable (69). Hot water treatment, suggested in 1919 (56), was worked on further for black leg (70). It was reported to be effective for black leg and black rot in 1924 (16, 18). It has since that time remained the standard disinfectant for seeds of cabbage and other crucifers.

The chief seed-borne pathogens of common bean (*Phaseolus vulgaris* L.) are the bacterial blight organisms (*Xanthomonas phaseoli* (Smith) Dowson; *Pseudomonas phaseolicola* (Burkholder) Dowson), the bacterial wilt organism (*Corynebacterium flaccumfaciens* (Hedges) Dowson) and the anthracnose organism (*Colletotrichum lindemuthianum* (Sacc. & Magn.) Briosi & Cav.). Extensive experiments were reported (55) in 1917 to devise means of disinfecting bean seeds. The results were entirely negative. Attempts of others since have failed to yield a satisfactory treatment for bean. The same holds true for the bacterial blight of lima bean (*Pseudomonas syringae* van Hall) and for the important seed-borne pathogens of pea (*Mycosphaerella pinodes* Berk. & Blox.; *Ascochyta pinodella* L. K. Jones; *Ascochyta pisi* Lib.; *Pseudomonas pisi* (Sackett) Dowson). Several of the newer protectant dusts partially disinfest pea seed of surface-borne *Ascochyta* (51), but for complete control seed disinfection is required, and no suitable means has been forthcoming.

Beet-seed treatment has been the subject of much investigation both in United States and in Europe since early in this century. The most common seed-borne pathogen is *Phoma betae* (Oud.) Frank; others are *Peronospora schachtii* Fckl., *Cercospora beticola* Sacc. and *Pseudomonas aptata* (N. A. Brown & Jamieson) Stapp. Coons and Stewart (22) in 1927 showed that hot water at 60°C. for 10 minutes disinfects seed infected with *P. betae.*, but the nature of the protective pulpy tissue surrounding the seeds in the seed ball made liquid treatments unsatisfactory and impractical. Shearing of seed clusters to free individual seeds is a relatively recent process which obviates the difficulty. Leach (49) found that Ceresan (2% ethyl mercury chloride), New Improved Ceresan (5% ethyl mercury phosphate), Arasan (tetramethylthiuramdisulfide) and dichloro-naphthoquinone controlled seedling infection by *Phoma* but did not completely eliminate the fungus. Ark and Leach (3) found that New Improved Ceresan, used as a dip or dust, and Arasan as a dust, were satisfactory disinfectants for *Phytomonas aptata.* In most sugar-beet and table-beet experiments the emphasis has shifted in recent years toward protection against damping-off fungi in the soil.

The work of Gardner (28) and that of Carsner (15) established the seed-borne nature of the organism of cucurbit anthracnose (*Col-*

letotrichum lagenarium (Pass.) Ell. & Halst.) and of cucumber angular leaf spot (*Pseudomonas lachrymans* (E. F. Sm. & Bryan) Farraris), respectively. Gilbert and Gardner (29, 31) developed seed disinfestation control by the use of mercuric-chloride solution, a practice still in vogue. The celery-blight organism (*Septoria apii-graveolentis* Dorogin) was shown to infect celery seeds, and the hot-water treatment devised in 1921 (48) has been used widely. Gardner and Kendrick (30) showed that the causal organism of bacterial spot of tomato (*Xanthomonas vesicatoria* (Doidge) Dowson) is seed-borne, and they worked out a mercuric-chloride treatment reported in 1921. Formaldehyde solution with or without previous immersion of the seed in alcohol was also effective as a seed disinfestant. More recent work has brought forth New Improved Ceresan (5% ethyl mercury phosphate), applied as a dust or a drench as a seed disinfestant (24, 68). Development of bacterial canker as a major disease in many areas in the United States following 1926 (13) indicated that the organism (*Corynebacterium michiganense* (E. F. Sm.) Jensen) is commonly seed-borne. Chemical treatments failed to rid the seed of the organism. Blood (6) in 1933 reported that if seed was allowed to remain in fermenting pulp for 72 hours it became disinfected. He attributed this to the increasing acidity of the pulp. He worked out an acetic-acid treatment which is effective if applied to freshly extracted seed (7). At the moment inspection and certification of seed fields together with application of New Improved Ceresan (5% ethyl mercury phosphate) as a dip or as a dust disinfestant are the chief measures applied to tomato (24, 28). *Verticillium* spp. and *Fusarium oxysporum* f. *lycopersici* (Sacc.) Snyder & Hansen and *Pellicularia filamentosa* (Pat.) Rogers are also occasionally seed-borne. Hot water (30 minutes at 50° C.) is the only disinfectant so far devised (5*a*.) Pepper and eggplant seed may be considered in the same category as that of tomato.

From about 1900 to 1925 major attention was directed toward treatment of vegetable seeds to disinfest or disinfect them. Fairly satisfactory but usually cumbersome methods were devoloped for cabbage, tomato, celery and cucurbits. Little or no progress was made with fleshy seeds, such as pea, lima bean and bean. Up to 1925 seed protection received only minor attention, the notable example being the formaldehyde-drip control of onion smut. It was from 1925 to 1932 that the field of vegetable seed protectants was opened up (17, 22, 34, 37, 43, 57).

Coons and Stewart (22) reported in 1927 on an extensive series of treatment experiments with sugar-beet seeds. They emphasized that the major damage to seedlings in the period immediately following germination is not usually due to *Phoma betae,* which commonly infects beet seeds, but to soil-inhabiting fungi. They pointed out that complete disinfection of the seed by hot water at 60° C. for 10 minutes has no effect upon soil-inhabiting damping-off fungi. They found that mercury and copper compounds applied as dusts has value as seed protectants. Clayton (17) in 1928 showed marked improvement in stands with a number of vegetables when seed was treated with organic mercury compounds, Semesan, Uspulun and Bayer Dipdust, applied in either liquid or dust form. Jones (43) reported in 1931 that organic mercury dusts (Uspulun and Semesan) are effective protectants of pea seed and are particularly useful in wet soil in which soil inhabitants cause pre-emergence damping off. Horsfall began to explore the value of other seed protectants on various vegetables shortly before 1930. Poor adherence of copper sulfate powder was a decided handicap. Cuprous oxide was found to be a cheap, effective protectant for peas and tomatoes.. First used as red cuprous oxide and later as the more finely divided yellow oxide, it came into extensive use on peas. Zinc oxide was also found to be an effective protectant and was used extensively on spinach (20, 21, 40). Several disadvantages of cuprous oxide and zinc oxide soon appeared. Both increased the friction between seeds in the planting operation, as was found earlier with copper carbonate as a wheat-seed-treatment dust, and it became necessary to add graphite to lubricate the seed in machine planting. Cuprous oxide was found to cause some injury, and it was particularly injurious to crucifer seeds. Zinc oxide caused injury on peas (38, 39, 41, 59).

The search for less phytotoxic materials continued and was stimulated by the shortage of mercury and copper which developed with the onset of World War II. Non-metallic organics were explored, and several new seed protectants were uncovered. The first of these was tetrachloro-para-benzoquinone, later marketed as Spergon. It was reported as an effective protective on lima beans in 1940 (23) and on peas in 1942 (26), and was rapidly adopted as a dust treatment for both. In 1943 tetramethylthiuramdisulfide (Arasan) and ferric dimethyldithiocarbamate (Fermate) were reported as effective protectants in several parts of the United States (25, 32, 53, 60, 65).

Arasan has been adopted widely as a protectant on sweet corn, peas, onion (pelleted), lima beans and other vegetables. To a large extent Spergon and Arasan have now replaced cuprous oxide and mercury dusts on vegetable seeds. Spergon has been found, however, to be inferior on beet and spinach, and not as consistently effective on sweet corn as Arasan. Other organic materials coming into use as seed protectants are 2, 3 dichlor 1, 4 naphthoquinone (Phygon) and zinc trichlorophenate (Dow 9B).

In the development and application of seed protectants certain problems arise which are of less importance with seed disinfestants and seed disinfectants. In the last two types of treatment the environment at the time of treatment can be controlled, and the chief variable is the physiological status of the seed sample concerned. In the case of seed protectants the environment is usually beyond control and may be expected to vary widely as to soil type, soil reaction, soil moisture, soil temperature and soil flora. Buchholtz (14) has shown, for instance, that on sugar beets in Iowa, *Pythium debaryanum* Hesse is the major soil fungus which attacks seeds and young seedlings. It is quite successfully controlled by seed protectants. However, another damping-off fungus, *Aphanomyces cochlioides* Drechsler, begins to attack seedlings about the time when Pythium injury ceases, and this fungus is not controlled by seed treatment.

Since treatment may be highly successful in one instance and of much less value in another, uniform procedures for general use as to material and dosage are more difficult to define for protectants than for disinfestants and disinfectants. In recognition of this fact, a special committee of the American Phytopathological Society, under the leadership of Dr. H. T. Cook, undertook on a national scale a series of seed-protectant-treatment tests on standard vegetables with promising fungicide dosage rates. These trials were extended over a period of five years beginning in 1941. In this plan the same lot of seed was treated and distributed to various locations in the United States and Canada for trial. Thus the seed variable was eliminated and attention was focused upon variables in the soil environment which affected the usefulness of the fungicide concerned. In this way some fungicides were eliminated or placed in secondary positions for certain seeds, and better evaluation of dosages was secured. Certain of these results are now available in published form (1, 2). They have comprised a base upon which recommendations were made during the World War Period.

It is indeed surprising that such general agreement was reached in these extensive cooperative experiments with seed protectants, when trials were conducted under such a wide range of conditions. While they leave some important questions unanswered, they provide a tentative basis for application and a point of departure for further exploration and research. Crucifer seeds are sensitive to copper compounds, while beets and spinach are commonly injured by Spergon. The latter is eminently successful as a protectant on peas and lima beans. Under certain conditions, Semesan Jr. (1% ethyl mercury phosphate) and Spergon are eminently successful protectants for sweet corn. The former has the additional advantage of being a disinfectant when *Diplodia* spp. are concerned. To injured seed it is, however, phytotoxic under some conditions. Since Arasan has been shown to be a safer protectant and a fairly efficient disinfectant, it is rapidly becoming the most widely used fungicide for corn seed. Leach and Smith (50), in a critical study of seed protectants on peas, secured beneficial results with soil infested by *Pythium ultimum* Trow. but not with soil infested with *Pellicularia filimentosa* (Pat.) Rogers (*Rhizoctonia solani* Kühn). Semesan, cuprous oxide, Spergon, New Improved Ceresan and Arasan were very effective, the first two being the most so. However, when germination was delayed by low soil temperature cuprous oxide and New Improved Ceresan (5% ethyl mercury phosphate) were injurious to the extent that germination was retarded and depression in yield resulted. Taylor and Rupert (64) showed that Arasan is distinctly superior to Spergon and cuprous oxide in the duration of the protective period, and thus is most effective in reducing post-emergence damping off. Their tests were made with spinach planted in soil inoculated with *Pythium ultimum.*

Foster (27) offered an explanation for the fact that copper compounds tend to be injurious to seeds of crucifers and peas and sometimes to cucumbers, while they are not so to seeds of beet, spinach and solanaceous vegetables. In fact, he showed that copper sulfate and cuprous oxide in certain concentrations actually stimulate the latter group. He suggested that the differential effect of copper on seeds may be due in part to its effect upon the enzyme system in the seed. One of the systems present in pea and cucumber seeds requires the presence of sulfhydryl compounds, and enzymes of this type are inhibited by copper. In beets and spinach, on the contrary, the enzyme system contains tyrosinase which requires the presence of copper ions for its activity.

CURRENT RECOMMENDATIONS

In the table on page 597 are listed some of the currently accepted methods of seed treatment which are worked out for disinfection, disinfestation and protection, respectively.

FUTURE NEEDS IN SEED TREATMENT RESEARCH

Exploration for new treatment materials since 1933 has yielded a small number of compounds vastly superior to those previously available as protectants for vegetable seeds. In this period we have seen the fungicides containing the heavy metals (*e.g.*, cuprous oxide and zinc oxide) rise and recede in favor largely because of their phytotoxicity, while organic compounds of sulfur and chlorine have come to the front, *e.g.*, Spergon (tetrachloro-para-benzoquinone), Arasan (tetramethylthiuramdisulfide), Phygon (dichlornaptho-quinone). None of the last group is without limitation. Research in the nature of the interaction of each with host, pathogen and environment is the greatest present need. Exploration for better compounds may thereby become less empirical.

In the same period of nearly two decades methods of seed disinfection have changed little. Only in the case of sweet corn do seed protectants serve also as seed disinfectants. In the case of seeds of crucifers, celery, tomato, pepper, eggplant and beet we have nothing to supplant hot-water treatment. Its effectiveness is offset by its clumsiness and by a narrow margin of safety. Most seed producers and seed merchants, consequently, avoid it. McWhorter and Miller (54) have explored the possibility of heated vapor as a substitute for the hot-water soak. With fleshy seeds (beans, lima bean, and pea) no advance has been made. Here, certainly, plant pathology has been at a standstill for a quarter century and shows little sign of moving off dead center. In the meantime the pea

Vegetable	Disinfectant	Disinfectant[a]	Protectant[a]
Beet	Hot water, 60 % 10 min.	New Improved Ceresan dust,[b] 0.5%	Arasan,[c] 0.5%
Cabbage, cauliflower and other crucifers	Hot water, 50° C., 30 min.	None	Arasan, 0.5%
Carrot	Hot water 52° C., 10 min.	None	Arasan, 0.75%
Celery	Hot water, 48° C., 30 min.	None	None
Cucumber, and other cucurbits	None	Mercuric chloride, 1–1000, 5 min., rinse	Spergon,[d] 0.3% or Arasan, 0.3%
Lettuce	None	None	Spergon, 2.0%
Lima bean	None	None	Spergon, 0.1%
Onion	None	None	For damping off: Arasan, 0.5% For smut: 1–64 formaldehyde, 1 gal. per 350 feet of row; or Arasan, 25% pelleted on seed with Methocel sticker
Pea	None	None	Spergon, 0.2%; or Arasan, 0.2%
Spinach	None	None	Arasan, 1%
Sweet corn	None	Arasan, 0.3%	Arasan, 0.3%
Tomato, pepper, eggplant	Hot water, 50° C., 30 min.	New Improved Ceresan dust, 0.5%	New Improved Ceresan, dust, 0.5%

[a] The dosages of dusts are given as percentages of the weight of seed.
[b] 5% ethyl mercury phosphate.
[c] 50% tetramethylthiuramdisulfide.
[d] Tetrachloroparabenzoquinone.

crop is at the mercy of *Ascochyta* blights and bacterial blight, and the bean crop is continually pest-ridden with bacterial blights. Production of disease-free seed of these crops in any sizeable amounts is far from reality.

In fact, the great emphasis on seed protectants has, if anything, tended to deemphasize seed disinfection and its importance. With the wholesale application of dust protectants (usually worthless as disinfectants) to seeds at the source of distribution, there is a growing tendency for layman consumers to assume that all seed-borne pathogens have been cared for. Moreover, there is a striking lack of warning in current extension literature against this illusion. Seed salesmen are known to advise customers that hot-water treatment is not necessary for cabbage if it is dusted with Semesan, and that cucumber seed need not be treated with mercuric chloride for angular leaf spot if dusted with Semesan, *etc.* What is the result? Nearly every year in the last several the writer has encountered epidemics causing losses running into thousands or tens of thousands of dollars due to pathogens traceable directly to introduction on infected seed.

What is the answer? If plant pathology is to meet this important gap in disease control, one or both of two developments must come about. First, adequately supported research must be directed toward the improvement of methods and materials for seed disinfection which are sufficiently safe and effective so that seed producers and distributors can be expected to use them at the source. Second, research must be directed toward the development of bio-assay methods whereby presence of important plant pathogens can be detected with certainty. The latter is a tool much needed by seed producers and examiners and is one which is essential to an adequate system of regulation and certification. Until research shows definite progress in these directions vegetable crop losses from pathogens which infect the seeds may be expected to continue unabated.

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HISTO-PHYSIOLOGICAL GRADIENTS AND PLANT ORGANOGENESIS

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CHAPTER I. GENERAL CONCEPT OF A SYSTEM OF GRADIENTS IN LIVING ORGANISMS

The Concept of Gradients in Physics; its First Applications to Biological Sciences

A gradient is the progressive variation of a factor in function of the position. For instance: along a metallic rod, heated at one extremity, a temperature gradient appears; when pure water is poured gently on a sugar solution, a gradient of sugar concentration is established in the mass.

Application of this concept to biological sciences was made by Boveri (14, 15) who distinguished a progressive chemical variation in the cytoplasm of *Ascaris* eggs. Child and his collaborators (20) expanded the idea by recognising a "gradient of metabolism" along the embryo axis. Many embryologists (20, 23, 37, 46, 81, 90) subsequently enlarged the idea, and the concept of an embryogenic field was introduced. As Huxley (46) states: "Within these fields various processes concerned with morphogenesis appear to be quantitatively graded, so that the most suitable name for them is field-gradient system or simply gradient-fields". We shall examine here the application of these concepts to the study of plant organogenesis.

Classification

In the body of a plant, gradients may be classified as follows (71):

a) Physico-chemical gradients, referring to physical or chemical elements, such as temperature, osmotic pressure, pH, rH, water concentration, glucose concentration, *etc.*

b) Physiological gradients, concerning the functions of tissues, *viz.*, respiration, photosynthesis, growth rate, tropisms, *etc.* They indicate the changes of intensity in these functions, according to the location in the plant body.

c) Anatomical and histological gradients, which concern variations in dimensions, nature and shape of cells and cell-groups. Some of them are quantitative, others qualitative. We shall reserve the term "gradation" to designate complex variations which are the sum of many elementary gradients. For example, a chemical gradation may involve a series of gradients concerning the concentrations of carbohydrates, of oils, *etc.*

Expression of Gradients in the Different Systems of Coordinates

A stem produces roots at its base, leaves and flowers at its summit, thus displaying a polarity which involves an intricate set of axial gradients (11, 18, 42). Such modifications do not occur, however, only in a one-dimensional system. In a ribbon-shaped organ, for example, a grass leaf (67), there are definite variations in its structure and functions not only along its length but also in its width, the latter chiefly in relation to veins. Thus we must recognize a set of transverse gradients in addition to the axial ones. Together they constitute a two-dimensional field, governing all development and functions of the tissues. We may trace, in such a field, a network of isopotential lines (lines of equal properties) which at every point are perpendicular to the direction of the gradients concerned.

Furthermore, an organ possesses not only length and breadth; it also has thickness, which involves another group of gradients. We are thus obliged to recognize a tri-dimensional field, with series of isopotential surfaces, always perpendicular to the vectors expressing the gradients in space.

If an organ is cylindric, as is generally so of stems and roots, it may be useful to employ, in place of the usual three axes, a system of polar co-ordinates. Thus, besides the axial gradients, a set of radial ones can be distinguished (Fig. 7, II; 8, VIII, IX).

Interactions Between Gradients

After having separated the elementary gradients we must always bear in mind their re-synthesis, for all gradients are merely diversified expressions of the progressive variation of the properties and of the functional equilibrium of living matter in the mass of cells constituting the plant. It is easy to conceive that they are all interdependent, directly or indirectly, the slightest modification in any one of them having unlimited possible repercussions on all the others.

The physiological gradients appear, to a certain extent, to govern the whole system. In the meristem of an embryo, the establishment of metabolic, mitotic and respiratory gradients is noticeable during the earliest stages of growth. These gradients give birth immediately to the two other categories, *viz.,* physico-chemical gradients, resulting from localization of the products of their activity (oxygen concentration, rH, carbonic acid, pH, *etc.*) and anatomic-histological gradients resulting from their influence on the development of the young cells.

Reciprocally, these induced gradients react on those that gave rise to them, for the local concentration of any product, *e.g.,* carbonic acid or auxin, modifies all the functional gradients. Furthermore, when a group of cells has differentiated in a given way, its specialized activity necessarily transforms the entire system of physiological gradients as well as the chemical ones. Such interactions may be represented thus:

$$\begin{tikzcd}
\text{Physiological gradients} \arrow[dr, shift left] & \\
& \text{Physico-chemical gradients} \arrow[ul, shift left] \arrow[dl] \\
\text{Histological gradients} \arrow[uu] &
\end{tikzcd}$$

CHAPTER II. PHYSICO-CHEMICAL GRADIENTS

Chemical Gradations

Pigment gradations. Some chemical gradations, revealed by variations in color, are readily seen. For instance, in *Melampyrum nemorosum* (74) there is a progressive transformation along the stem, affecting both foliar morphology and coloration (Fig. 1). The stem base bears ordinary lanceolate leaves, definitely green, while the apex shows intensely violet floral bracts provided with acute lobes. Every grade of transition can be observed between these extremes.

This transformation first appears in two differentiated spots at the base of the lowest leaves of the flowering portion of the stem

(Fig. 1, No. 3). These spots are noticeable by their translucency and by the indentations in the outline of the leaf provoked by their reduced growth. Upward along the stem, from one leaf to another, the differentiated regions occupy progressively greater and greater areas and become ever more deeply colored, first, in pink, then in violet. The last vestige of green persists at the extreme tip of the leaves, Nos. 10 to 15; in the uppermost leaves (from No. 16 up) all traces of green tissue have disappeared. With respect to growth, the length of the appendages decreases regularly from below upwards (Fig. 1, curve AB).

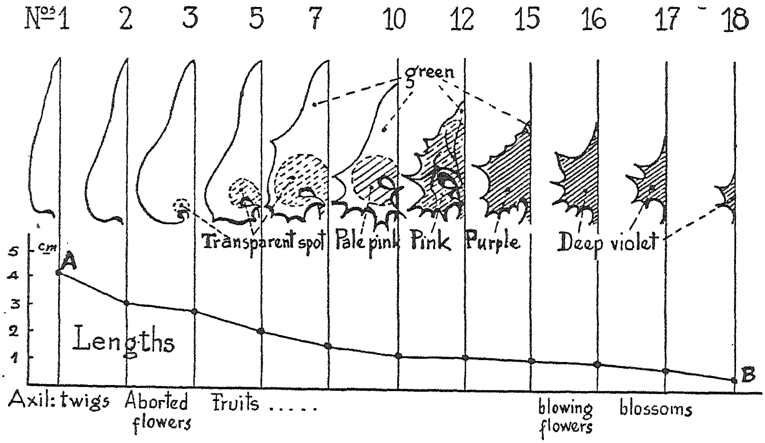

FIG. 1. Gradation of foliar morphology and coloration in *Melampyrum nemorosum* (74). The numbers refer to the leaves on the floriferous part of the stem. The leaves are figured without regard to their relative dimensions. Curve AB indicates their lengths in centimeters; hatched areas represent anthocyanin regions.

In *Melampyrum* we thus observe a conflict between two modes of growth and development of the tissues. The first concerns the vegetative form of the appendage and leads to the formation of a comparatively thick, opaque organ, rich in chlorophyll, lanceolated and entire. The second concerns the bracteal state and leads to a thin, translucent organ, poor in chlorophyll, producing abundant anthocyanins, tending towards a general pentagonal form with numerous acute lobes; it involves inhibited growth, chiefly in the longitudinal sense. This second form of evolution of foliar meristems operates under the influence of reproductive organs in the course of their development.

The close association of these two foliar types and the gradual replacement of the first by the second are clearly the result of the fact that production of young leaves is a progressive and rather slow process, whereas the sexual crisis is a sudden, rapid phenomenon. When flowering occurs, the varous leaf-primordia are struck by this crisis at different stages of development. Those at the base of the stem have already reached almost their definitive shape and are therefore affected only in a minute portion of their body, *i.e.,* in the undifferentiated cells persisting at the base of their blades. The leaf primordia at the apex of the stem, on the contrary, are still entirely in a meristematic, *i.e.,* versatile stage when the floral crisis occurs. Thus every part of them is affected by the deep change of metabolism provoked by flowering, and their whole body develops into an appendage of the second type.

Other genera, *e.g., Poinsettia, Nidularium, Amaranthus,* exhibit similar colour gradients associated with morphological ones, thus clearly showing chemical gradations. They include many decorative species, remarkable for the splendour of their bracteal system.

Gradations of essential oils. In some perfume plants, such as *Rosa* and *Jasminum,* the fragrant essence is localized in the flower, constituting a secondary sexual character just as do the pigments in the previous cases. In others, as in the Labiatae, it is elaborated by stems and leaves in specialized secretory hairs. But, even then, it is not indifferent to the sexual influence. For example, in *Lavandula* the fragrance is more delicate near the flowers. In orange tree the perfume is entirely different in the leaves, in the petals, in the fruit pericarp and in the vesiculous hairs of the endocarp. It would thus be necessary to distinguish here not only one but a set of gradients concerning each of the odoriferous essences, all of them being under the influence of the reproductive process.

In some cases complex secretory gradients can be detected, even in minute groups of cells. For example, the glandular organs studied by Martens (57) in *Polypodium virginianum* secrete tannic products in their basal cells and resins and essences in their apical ones. In the epidermis of grasses such tiny chemical gradients, on a cellular scale, (67) will be pointed out later.

Detailed study of essential oil gradients would be of great value for the perfume industry, a difficult task considering the fugacious and unstable character of these products.

Gradations of carbohydrates. Colin (21) and his students have carefully investigated the variations of carbohydrates in different plant organs. For example, in sugar beet, at the end of the day, the foliar parenchyma is rich in starch and sucrose. Toward the base of the big veins and in the petiole the sucrose concentration decreases, but, in compensation, glucose and levulose (fructose) increase steadily (Fig. 2, I). At the base of the petiole, sucrose practically disappears; but, a short space lower, in the tuberized root, its concentration abruptly rises up again, while glucose and levulose almost vanish. The transformation occurs in a surprisingly short distance, within a few layers of cells (Colin). Thus, once more, we observe the two kinds of gradients already seen in pigments and oils, *i.e.*, slowly progressive or abruptly broken.

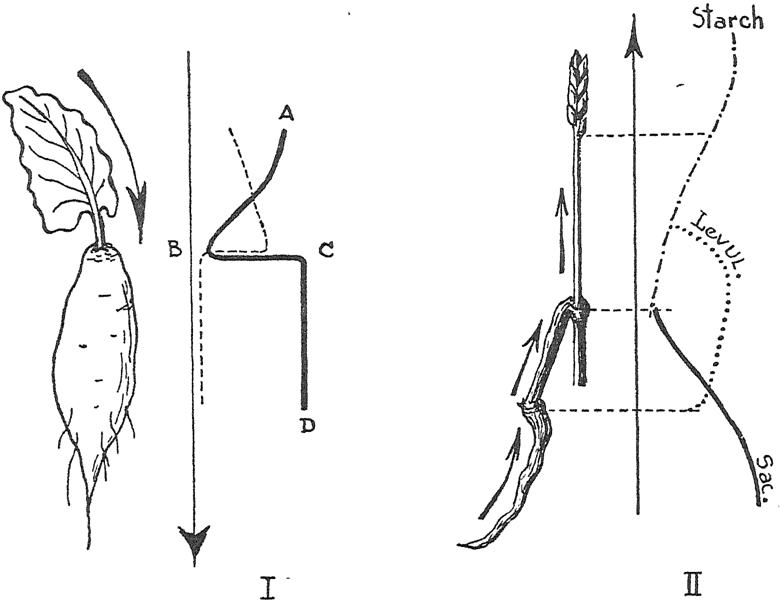

FIG. 2. Gradients of carbohydrate concentration (21). I: sugar beet; II: wheat. Solid curves indicate sucrose concentration; dotted lines, levulose concentration. Arrows show the direction of migration. In II the leaf sheath is extroversed to make the diagram clearer.

These gradients are modified acccording to the season and related to growth phases. Leonard (49) notes that a high level of fructose corresponds to rapid growth. In potatoes there is great increase of starch concentration in the tuberized portion, and in carrots there are also significant carbohydrate gradients in correlation with bud and root development (62, 63). When buds are present, the carbohydrates of secondary tissues are translocated so as to produce new roots with well organized primary structure. When buds are sectioned, the tendency is toward an increase of old secondary tissues and formation of new secondary structures.

Not less interesting is the carbohydrate gradation along a stem during maturation of seeds. For example, in wheats (Fig. 2, II) the starch percentage increases toward the summit of the terminal internode and becomes maximum in the grain. In compensation the percentage of levulosans, which increases downward along the leaf-sheath, decreases during the ascent along the internode. Careful study of these gradients may lead to important agricultural applications.

Gradations of alkaloids. Chase (19) has observed notable variation in the distribution of nicotine in the organs of tobacco plants. This alkaloid does not exist in the seed, except in the integument, but, as soon as germination begins, it appears. In young seedlings, three millimeters long, it is present in the growing points, in the hypocotyl and in the root, chiefly in the piliferous layer. In the stem cortex the alkaloid percentage decreases, first from the outside inwards, then increases again in the pith. On the leaves there is considerable exsudation of nicotine by hairs and stomata, chiefly around the ends of veins. We thus note in tobacco a definite set of chemical gradients, both axial and transverse, and their modifications in function of the age of the tissues. They are correlated with ionic and oxido-reductive conditions of the cells.

Ionic Gradients

In transverse sections of stems and roots, Priestley (76, 77) noticed the establishment of gradients of pH, the xylem being acid and the phloem relatively alkaline (Fig. 3, A). Between them there is a zone of intermediate pH, possessing the most favorable conditions for the conservation of meristematic activity. In this zone the protoplasmic proteins, their environment being near their isoelectric point, show a minimum tendency to swell and to combine with salts (89). This explains the position of undifferentiated, non-vacuolate cells of cambium between xylem and phloem and their peculiar mode of division. Their partition walls are likely perpen-

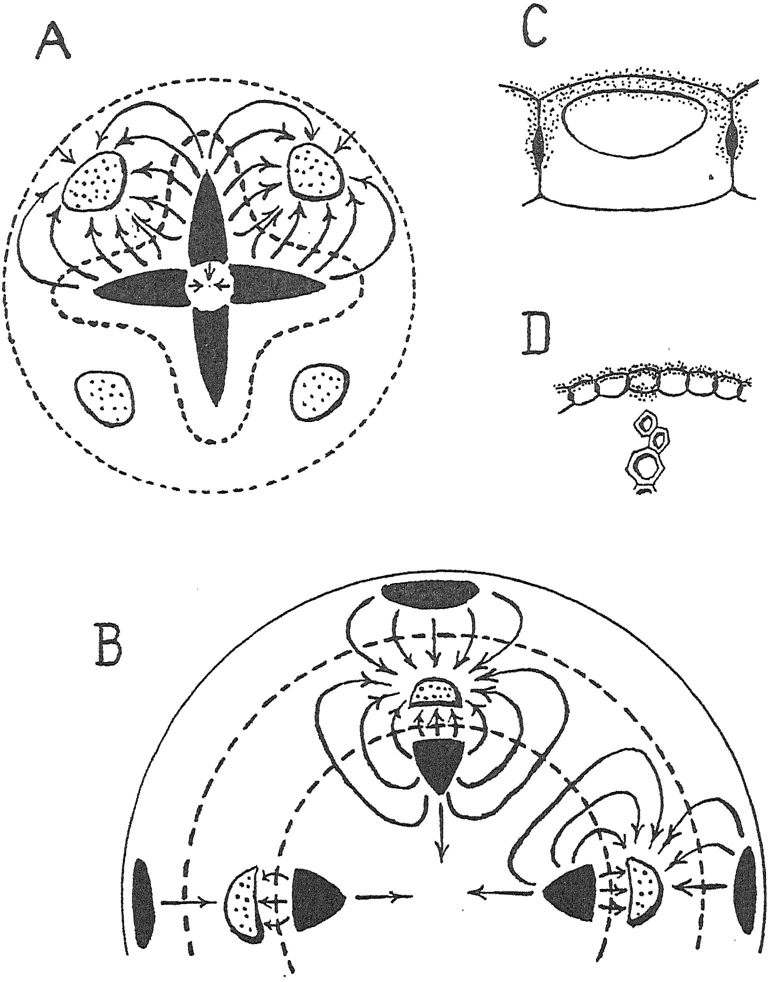

FIG. 3. A: Disposition of ionic gradients in the transverse section of a young root (76). Solid black areas indicate acidic zones (xylem); dotted areas, alcaline zones (phloem). Arrows show directions of increasing pH (hydrionic gradient). Dotted lines indicate levels of intermediate pH where cambial activity is preserved. B: The same conception applied to a young stem. Sclerenchyma is figured, as xylem, in solid black. C: Oxidation gradients in the endodermis (85). Oxidase action, shown by stippling, is more intense on the outer tangential wall, and, in young stages only, on the Casparian strip. D: On a "gap cell" in front of a xylem pole oxidation is more intense and invades the inner wall.

dicular to the direction of hydrionic gradient. The inner daughter-cells are soon vacuolate, on account of their being in a zone of lower pH, and they develop into xylem elements. On the contrary, the outer daughter-cells, living in an alkaline zone, mature into phloem. Pericycle is also a zone of intermediate pH, whence its peculiar aptitude for cell proliferation.

It is highly probable that pH is among the leading factors governing the development of cells. Cork and sclerenchyma represent, as does the xylem, regions of low pH; thus the position of the subero-phellodermic layer is determined in the same way as that of the vascular cambium (Fig. 3, B). The multiple vascular layers of the Salsolaceae might be due to the establishment of a multinodal radial gradient of pH within the stelar parenchyma, with alternating acidic, neutral and alkaline zones disposed in concentric or spiral cylinders.

Besides these radial ionic gradients we can observe also axial ones. Along the culm of Indian corn, Gustafson (38) has shown a gradient of pH increasing from the base to the summit in the leaves and decreasing in the stem. Priestley notes also (76) that cell division is more rapid in the inner mass of the root meristem, which is more acid and which therefore "adds more cells to the stele than to the root cap".

Electric Gradients

Electric gradients have been detected in plant organs. Lund has shown that the apex of Douglas fir and White fir (51) is positive by comparison with the tip of secondary branches (longitudinal gradient); that the central cylinder is positive in regard of cortex (radial gradient). He states: "A definite but complex system of internal electric potentials exists in the tree, which must set up a corresponding system of internal electric currents. The general direction of such a system of currents appears to be upward in the wood axis and downward in the cortex. . . . Changes in P.D. in one region of the tree will therefore instantaneously modify the electric conditions in other adjacent or distant parts of the tree system".

These electric gradients are independent from light, therefore from photo-synthesis, and also from transpiration. They persist in the stela when cortex is removed or branches cut; they are modified by changes of temperature and by mechanical actions.

They are greater in actively growing regions; as electric resistance is lower in these points, the intensity of produced currents is thus maximum in young tissues. It seems that these P.D. can be correlated to ionic and redox gradients (11).

Electric gradients have been observed also in algae (10). Their modification by experimental application of electric currents (80) induces great changes in organogenesis (for example: rhizoids more numerous) and histological structure (for example: migration of chromatophores, electrophoresis of pigments).

Oxidation-Reduction (Redox) Gradients

The course of cell development is under the influence not only of ionized hydrogen concentration, but also of many other factors, particularly the oxidation-reduction potential in the environment. Van Fleet (84–86) has recognized that the outer tangential wall of endodermal cells is the seat of intense oxidation, correlated with the presence of unsaturated fats, and that the inner walls are endowed with reducing actions. Between these two opposite influences the radial walls are the seat of polymerizations, whence the formation of Casparian strips characterizing endodermal structure.

Thus the endodermis appears as a point of rupture in the rH gradient, just as the beet collar marks a rupture in the gradient of sucrose. In plant anatomy the differentiated layers appear as the tangible expressions of critical levels in physico-chemical gradients.

Interactions between the hydrionic and redox gradients are noticed by Van Fleet (86) who states that: "Neutral or alkaline conditions associated with the phloem result in the inactivation or release of inhibitols and additive compounds from the fats, and oxidation is accelerated". On the contrary "the acidic zone of the xylem depresses oxidation". This fact suggests some explanation of many features of plant anatomy, *e.g.*, the appearance of Casparian strips in stems grown in darkness where alkaline conditions prevail and oxidations are magnified; and the presence of "gap cells" in front of xylem and generally in acid zones where unsaturated fats are inactivated (86). These characters are closely related to the behaviour of tissues toward water.

Hydration Gradient

The water content at different levels of a plant is governed in first place by the absorption gradient of its integument. From its youngest stages a seedling shows in this regard a polarized structure, the radicle constituting the absorbent pole, the gemmule the transpiratory one. Between them there is a basipetal absorption gradient and an acropetal transpiration gradient. Both are factors in the ascent of sap and depend on the internal conditions of the plant as well as on the external factors of soil and air. Only the young parts of the root have an absorbing role; between them and the transpiration system (leaves and young stems) there is a passive zone where both functions are practically reduced to zero, namely, the old parts of roots and stems surrounded with cork layers.

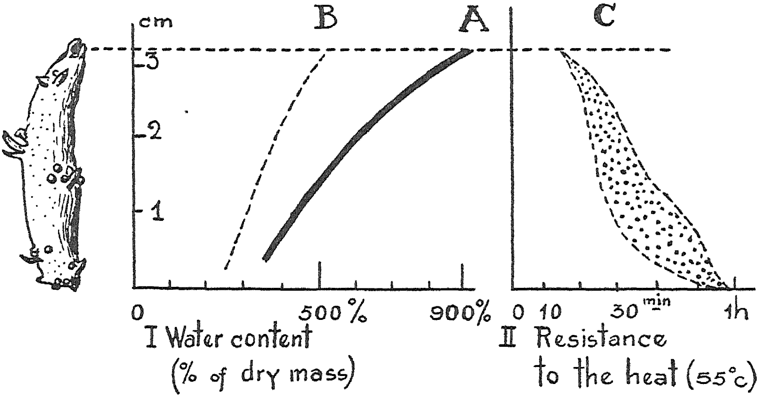

FIG. 4. I. Hydration gradient in potato sprouts (74). Abscissae show water content in per cent of the dry matter. A: In a turgescent sprout; B: In a sprout having suffered incipient desiccation. II. Gradient of sensibility of potato sprouts to heating (74). Abscissae show the time during which tissues are resistant to a temperature of 55° C. Breadth of dotted area shows the range of individual variations.

The balance between absorption and transpiration determines the total amount of water held in the plant, and the hydric affinity of each tissue determines the local distribution of this water. The result of these forces is the establishment of hydration gradients. For instance, a potato sprout (73) shows at its summit a water content of 900% (900 gr. of water for 100 gr. of dry matter), but only of 350% at its base (Fig. 4,A). If the sprout is dried, a rotation of the hydration curve can be observed (Fig. 4, B); the tip loses 45% of its water when the base loses only 20%. Thus the youngest and most hydrated portions of the tissues lose their moisture the more easily.

Besides this axial variation plant organs also show a radial hydric gradient. Gibbs and Scarth (33, 34), measuring the local water content in different parts of logs, found it lower in the center than at the periphery. They also noticed remarkable changes according to the season. In June the moisture gradient generally tends to diminish, while in July it increases. This is related to sap circulation.

Osmotic Pressure and Suction Gradients

Numerous authors have detected osmotic and suction gradients in various parts of the plant[1]. For instance, Ursprung and Blum (83) found a suction of 0.9 atmosphere in the piliferous layer of bean roots, 1.3 in the first layer of the cortex, 1.7 in the second, 2 in the third, *etc*. But in crossing the endodermis there was a sudden depression in the gradient. For this change Van Fleet (86) provided the hypothesis that: "The higher suction pressure on the cortical side of the endodermis is related to the more intense oxidation on this side of the tissue; and, therefore, the rate of water-passage from the cortex to the stele may be controlled by the unsaturated fat redox system and its rate of oxidation". According to the same author: "The gap cell opposite the xylem is construed to be a specialized water-absorptive cell, based on the slight but constant oxidation associated with this cell" (86). This is a further example of interrelationship between the diverse kinds of gradients.

In addition to these radial ones, we can also observe axial gradients of suction. Molz (59) noted an increase of suction from the base toward the summit of a stem and from the base toward the apex of the leaf vein. Such gradients are responsible, to a large extent, for sap circulation (40, 43, 52).

We must distinguish between the static gradient of suction, *i.e.*, the value just necessary to sustain the sap-column in the plant stem, and the dynamic gradient, *i.e.*, the suction required to raise the liquid. The second is highly variable, according to the hydric resistance of the tissues to be crossed. Along a well vascularized stem, it can be only one atmosphere by meter; but in non-vascularized tissues it may reach, as previously seen (83), a few tenths of atmosphere from one cell to the next, *i.e.,* the enormous value of one thousand atmospheres by meter. This, of course, is a theoretical evaluation, for in aerial species the non-vascularized portions of tissues are always small. In aquatic species non-vascularized tissues occupy larger areas, but their suction gradients are weaker.

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[1] The term "suction" is generally reserved to the expression $S = P - T$, the difference between the osmotic pressure P of the cellular liquid and the mechanical tension T (resilience) of the cell wall (58). A discussion on osmosis was given in this Review by H. Clyde Eyster some years ago (29).

CHAPTER III. PHYSIOLOGICAL GRADIENTS

Gradients of Metabolism

In any living tissue, from one region to another, the entire metabolic activity varies in quality as well as in quantity. On the side of catabolism there are variations in intensity of respiration, of thermogenesis, of intra-cellular fermentations, *etc.;* on the side of anabolism, variations in intensity of photosynthesis, in rate and nature of gluco-, lipo- and proteosynthesis, *etc.* All are dependent upon the state of hydration and upon the physico-chemical gradients (redox, ionic, *etc.*). It is well known that, by dehydrating a tissue, the metabolic activity can be inhibited and, thus, all the physiological gradients lowered (53).

The latter are greatly influenced also by sexuality. During the blooming period, intensity of respiration is greater in floral parts than in leaves, and greater in maturing stamens than in corollas. In *Arum* inflorescences the rate of respiration in male flowers may be ten times as great as in the female, and their temperature is higher. During flowering the respiratory and thermogenetic gradients are thus clearly directed toward the male organs, which are the most intense foci of catabolic activity. In an analogous way the sporangiferous parts of fern leaves show a more intense respiration than sterile regions.

A similar relation appears in the regions of a plant attacked by a parasitic organism. Maresquelle (56) mentions that stems infested by a rust mycelium show increased respiration as a result of their resistance against the invading pathogen.

All physiological gradients are dependent upon the distribution of diastases, oxidases, peptidases, *etc.* (4, 12, 13, 27, 78, 84, 86). We can not consider them all in detail, a task which would involve the entire field of plant physiology. We shall therefore examine only some processes concerning plant resistance against external influences, processes which involve, in themselves, a large series of functional gradients.

Gradients of Sensitivity to Heat and Desiccation

We have already pointed out that the diverse tissues behave in different ways with respect to loss of water. This diversity in behavior is correlated with a corresponding variation in the reactions of these tissues towards heat. Gain (31, 32) has shown that, in a seedling, destruction by heat begins at the tip of the radicle. Later, David (24–26) observed that, when a seed is heated, the plant which rises from it shows a necrotic zone at the end of the root. For my part (73) I have made the following observations. When a potato sprout is exposed to 55° C. for 15 minutes, its apex is mortally affected (Fig. 4, C); after return to ordinary temperature the other parts of the shoot resume growth. In exposures of greater duration larger areas are affected (Fig. 4, C), the sprout base appearing to be the last region of resistance. Upon exposures longer than one hour the entire sprout is killed. Individual variations are slight in the initial stages but greater in the final ones (Fig. 4). Thus, in a stem as in a root, the meristems are the most affected tissues. The gradient of resistance to heat is obviously correlated with the gradient of maturation (see Chapter IV); it is "cambiofugal". But it is not the only factor to be considered.

Pea seedlings exposed to 55° C. undergo the following alterations in their development. After a 15-minute exposure only the extremity of the root is killed; the stem remains alive, being only slackened in its growth, and dormant buds in cotyledons axils are activated. After exposures from 15 to 30 minutes, an increasing length of the root is affected, and only following longer exposures is the stem injured. The conclusion is that the tissues of the stem are more resistant to heat than those of the root. Besides the local cambiofugal gradient, we must therefore consider a general gradient, associated with the polarity of the plant and with the histological differences between root and stem.

In addition to this axial gradient of sensitiveness to heat, there is also a radial one. In a potato sprout (73) the histological elements first affected by heat are the younger parts of vascular bundles. In plants arisen from heat-exposed seeds (24) the cortex may remain alive and, by continued growth, break the stele to pieces, this latter region being selectively deteriorated by heat.

In order to separate the action of heat itself from that brought about by mere desiccation, I have performed comparative experiments in dry and in damp atmospheres. In both cases the same direction of gradient appears, but with different slopes. In dry air it is steep, the exposure durations necessary to kill the cells being widely different along the organ from one extremity to the other (72). In humid atmosphere, where the direct action of heat is alone present, the resistance is more uniform. There are thus two kinds of sensitivity gradients: one of thermic sensibility, slowly progressive; the other of sensibility to water loss, with an abrupt slope. Both are greatest in the youngest parts of the tissues.

Gradients of Resistance against Parasitic Organisms

Noël Bernard (7, 8) has shown the process of infestation by endotrophic fungi in the tissues of orchid seeds and seedlings. The mycelium, entering through the radical pole of the undifferentiated embryo, provokes the start of germination processes. It spreads into the root cortex of the young plant but seems unable to invade the active meristems and the stelar cylinders. Thus we can distinguish tissues which are receptive to the parasite and others which are resistant.

Concerning the yew-tree mycorrhizas, I have observed (66) that the fungus filaments, entering the root through the absorbing hairs (Fig. 5, I), spread in the middle regions of the cortex. When the endophyte reaches the endodermis its progression is abruptly stopped, the deepest bark layers seeming to offer an insuperable obstacle to its further advance. This fact is likely due to the high tannin content in the yew endodermis, a condition related to the peculiar redox properties of this layer. Thus in the transverse section of a yew root we can represent the gradient of resistance to the parasite in the manner shown in Fig. 5, II; first a moderate tolerance in the outer part of the cortex; then a high degree of receptivity, *i.e.,* an exceedingly low resistance, in the middle cortex levels; finally a steep rise of the resistance curve upon approaching the endodermis to reach an almost absolute immunity in this layer. This is sufficient to show that the gradients of resistance to parasitic organisms can offer exactly the same variety of curves as the others; in some parts there is progressive variation with a gentle slope; in others, abrupt changes with sudden rising.

Lengthwise the fungus tends to spread toward the root apex (Fig. 5, III) where it is checked upon approaching the active meristem (Fig. 5, IV).

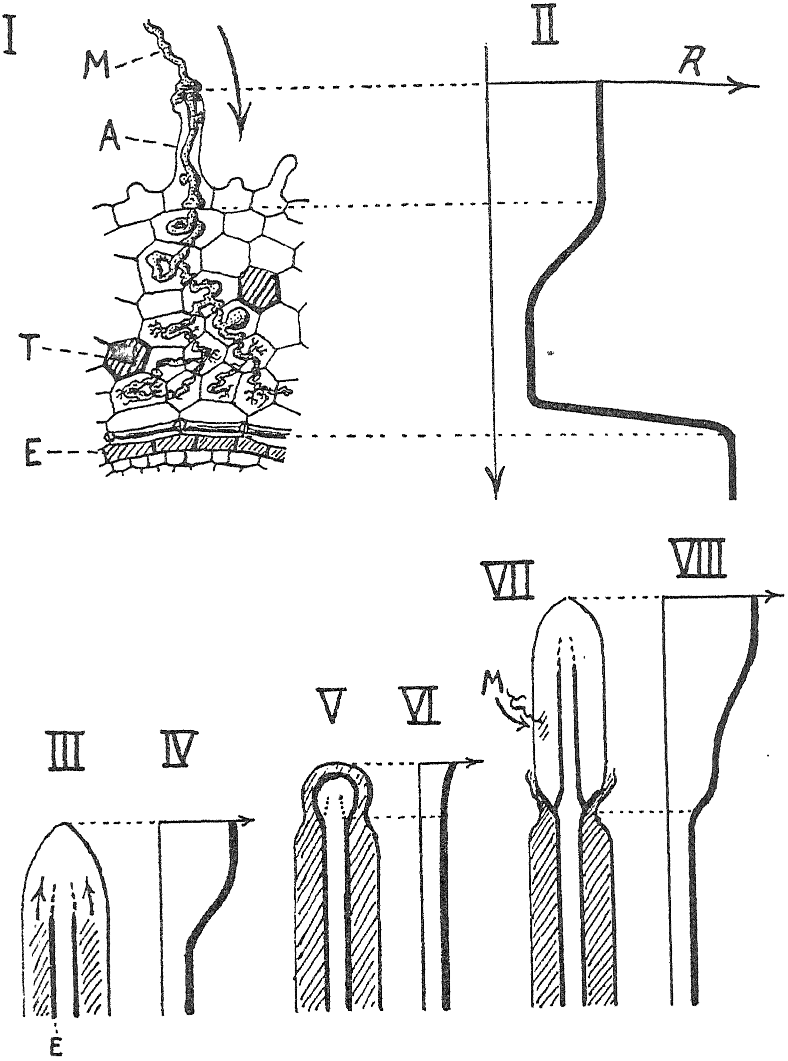

FIG. 5. Gradients of resistance of a yew root against endotrophic fungi (66, 74). I: Transverse section showing mycelium M entering through an absorbing hair A and spreading in the central cortex, except in tannin-containing cells T. The endodermis, E, filled with tannin, presents a barrier to the parasite. II: Curve expressing the relative resistance R of tissues in the same section (radial resistance gradient). III: Scheme of a yew root-tip in summer, in longitudinal section; infested zone hatched. V: The same in winter; endophyte has reached the apex but remains unable to cross the endodermis. VII: The same, next spring: a young root, issued by endogeneous process from the central cylinder, is first free from endophyte, but is secondarily reinfested (arrow M). IV, VI, VIII: Curves expressing, in longitudinal sections, the relative resistance of cortex tissues in each season (longitudinal resistance gradients).

These facts highlight the variety of devices displayed by plants to restrain infection. Physical barriers, such as thick cell-walls, are sometimes of little value; the mycelium may pass through them by applying to their surface a kind of sucker and emitting a boring point which digests its way through the cellulose membrane. Chemical barriers, on the contrary, are highly efficient, involving layers of cells filled with antiseptic products, such as tannic acid[2]. Other barriers are of a biological nature, involving a high capacity of the cells to produce digestive enzymes able to lyse fungal filaments (phagocytosis).

During winter, when meristems enter their phase of rest, their phagocytic activity is depressed, allowing the parasite to invade even the extreme tip of the root. The biological barrier is, at that time, ineffective, but the static chemical line of defence remains present, and, protected by the endodermis (Fig. 5, V), the central cylinder remains free from infection. The following spring a new segment of root issues from the upper part of the stelar tissues (66). Its birth is thus endogeneous, exactly like that of a lateral rootlet, with the only difference that this young segment has the same axis as the old one (Fig. 5, VII). This "sympodial" mode of growth acts as a means of preservation against infection. At first the new rootlet is free from parasite, as no direct connection exists between the old cortex and the new one. Infestation of this latter can originate only from fungus filaments penetrating from outside.

Thus the axial gradient of resistance is subject to remarkable seasonal variations. High near the root apex during summer (Fig. 5, IV), it declines in fall and winter (Fig. 5, VI) and again rises in young spring roots (Fig. 5, VIII). These circumstances are due to the metabolic nature of the factors governing this axial gradient. On the contrary, the radial gradient remains almost unchanged, the leading factor here being of a static nature and based upon the chemical content of the endodermis.

What was said concerning fungi is also true for resistance against bacteria. For instance, in root nodules of the Leguminosae, gradients of resistance, checking the progression of nitrogen-fixing bacteria and confining them in definite parts of the noduled root, can be clearly observed.

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[2] MacDougal and Dufrenoy (54) observe that: "decompensated respiration in pericycle and endoderm results in polymerization of the quinoids into gummy tannin masses, the presence of which forms a barrier to the extension of hyphae".

Concerning the viruses, it is well known that the mosaic of tobacco and the X-virus of potato plants are not transmitted to young plants raised from seed. Gratia and Manil (36) have explained this fact by showing that the viruses can spread only as far as the calyx of the flowers; that in the corolla their density is notably lowered; and finally, that, in stamens and pistils they are entirely absent. Therefore the seeds are conceived totally free from the pathogenic agent. We detect here an axial resistance gradient, probably of a chemical nature, acting within the flower complex of appendages. It is related to sexual activity, as is the first example we considered.

CHAPTER IV. AUXETIC GRADIENTS

Growth and Development

In the organogenesis of a plant, as in that of an animal, we must distinguish between two orders of phenomena, viz., growth, comprising only quantitative transformations such as mass increase of living matter or multiplication of similar parts (cells and organs), and development, comprising qualitative changes, with the emergence of new organs and functions. For instance, when a caterpillar increases in length and weight, it is a matter of growth; when it transforms into a butterfly, a matter of development. When a young plant enlarges and multiplies its leaves, growth only is concerned; when the first blossoms appear, a new stage of development is reached. Both examples involve passage from the vegetative state to the sexual one.

For each stage of development there is an appropriate set of needs and functional activities; each of them has its growth curves, and the same is true for the rate of all physiological functions. From one stage to another the entire gradient system of the plant is thoroughly transformed.

Gradients involved in plant growth are fundamentally of a dynamic, *i.e.*, physiological nature. But, since they are related to the physico-chemical conditions of the tissues (53, 99), and since their final result is the building of the permanent system of histological and anatomical gradations, we must place their study at the center of the entire subject. We shall group them under the title of "auxetic" gradients.

Growth of any tissue involves several activities: *a*) cell multiplication; *b*) cell-differentiation (first appearance of differences between previously identical cells); *c*) cell-enlargement (increase in volume by elongation); *d*) cell-maturation (thickening and modification of the membrane, chemical changes of the cell contents, *etc.*). The result is the "adult" state of equilibrium of the tissue, followed by senescence and, finally, by total death. (In the adult state some cells can be dead, while the others remain alive, as in wood, in epidermis, *etc.*)

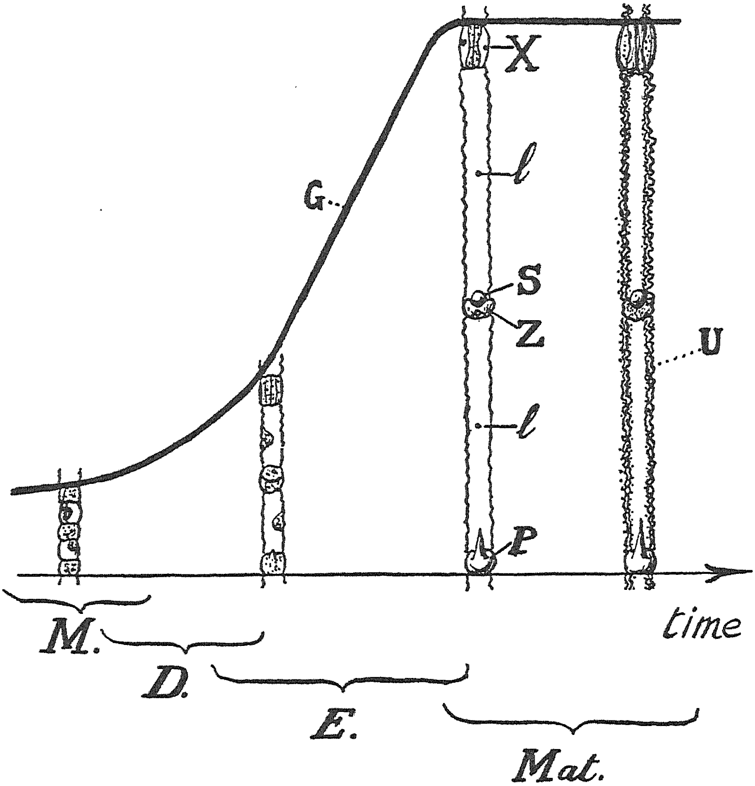

FIG. 6. Scheme of growth in grass epidermis (67–74). M: Stage of cell-multiplication. D: Stage of differentiation. E: Stage of elongation. Mat.: Stage of maturation of the cells. G: Growth curve. The represented cells are: X, of a stoma; l, two long cells of undulated type; S, a siliceous cell; Z, a cork cell; P, a hook; U, undulations of cell walls.

These stages are not strictly distinct; they encroach upon each other (Fig. 6). For instance, differentiation begins to appear while a few mitoses still occur, and maturation starts before elongation is fully achieved. But they correspond to distinct phases of cell activity and thus must be considered separately. They can be clearly distinguished, for instance, in the growth of the epidermal cells of a grass, wherein this tissue reaches the highest degree of differentiation (67):

a). In the meristematic regions, located at the bases of internodes and leaves, we find young cells in active division, all similar and roughly square-shaped (Fig. 6, M).

b). From the meristematic regions upwards we observe the birth of some differences between one class of cells, short and densely protoplasmic, and others lengthening and provided with large transparent vacuoles (Fig. 6, D). The cells of the second category do not divide but only elongate (Fig. 6, E). The first ones, on the contrary, undergo diverse kinds of mitosis and changes without being affected by elongation, in some cases the primitive short cell is divided[3] by walls parallel to the organ's axis into daughter cells which develop into a stoma (Fig. 6, X). In others a single wall appears, perpendicular to the organ's axis, and the short cell is divided into two superimposed daughter-cells whose destinies are dissimilar; one shows a granulate protoplasm which is progressively impregnated with colloidal silica (Fig. 6, S). It dies and becomes a small, hard and transparent structure, the shape of which is constant and characteristic in each species. The other one (Fig. 6, Z) undergoes suberization of its membrane, and its shape is moulded on that of the neighboring siliceous lump. The mutual relations between these sister-cells, forming a "silico-suberous couple", are remarkable; the siliceous cell S is always uppermost, nearest the apex of the organ; the suberous cell Z is always the lower. This indicates that a microscopic intracellular gradient, strictly correlated with the general polarity of the plant, had appeared in the mother cell prior to its division.

In other cases the apical daughter-cell develops by stretching its outer wall into a hair or hook (exodermic cell), while the basal sister-cell is suberized as previously. The primitive short cell may also offer no mitosis at all but evolve into either a siliceous, a suberous or an exodermal cell, to remain isolated amidst the neighboring long cells (Fig. 6, P).

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[3] Porterfield has observed in bamboos (65*a*) that the lateral cells of a stoma can develop from adjacent layers of tissue.

c). During the next stage, that of elongation, the short cells remain passive. Only the long cells take part (Fig. 6, E), and their length may be centupled in a short time, depending upon pH and auxin concentration. In exodermic cells the aptness to expand is concentrated in a limited area of the outer wall. If this wall retains such ability long enough, the cell becomes a hair. If, on the contrary, the tip of the protuberance is precociously sclerified or silicified, growth stops and the cell produces a spine.

d). The last stage, that of maturation, consists of changes within the cell contents and the cell walls, wherein the latter may acquire peculiar adornments, such as punctures, undulations (Fig. 6, U) and warts.

If we compare these phases with the general growth curve (Fig. 6, G), we see that cell multiplication and differentiation correspond to the initial period, where increase is slow; elongation is the period of rapid and constant rate of growth; and maturation marks the phase where growth reaches its end. The sigmoid shape of the growth curve can thus be analyzed (75).

Polarized Cell-Senescence

The foregoing discussion has shown the co-existence, in the same tissue, of rapidly senescent and dying cells (the siliceous ones) with others long retaining their ability to grow and to react toward auxins and physical agents. We can generalize from this and state that frequently when a cell divides, the two daughter-cells are unequally senescent, *i.e.,* their rates of evolution are different. This is apparent in every meristem, for instance, in the growing point of a stem (Fig. 7, I); from the cells engendered by the active divisions of the initials, those situated close to the apex remain physiologically young, *i.e.,* undifferentiated and keeping their totipotence, while those farther from the tip develop chemically, acquiring more and more specialized features and progressively losing their vitality. We can thus distinguish layers where senescence reaches its maximum, including the death of most component cells, such as wood and cork formations (hatched areas in Fig. 7); and other layers with persistent juvenility, such as vascular and subero-phellodermic cambiums (dot and dash lines in Fig. 7). Thus we can realize the existence of senescence gradients (arrows Fig. 7) progressing from regions of persistent youth towards those of decadent activity. A numerical expression of them will be given below.

This phenomenon, undoubtedly, is governed to a large extent by the establishment of physico-chemical gradients such as hydrionic, according to the conception of Priestly (76, 77), and redox, according to that of Van Fleet (84–86). Zones of persistent juvenil-

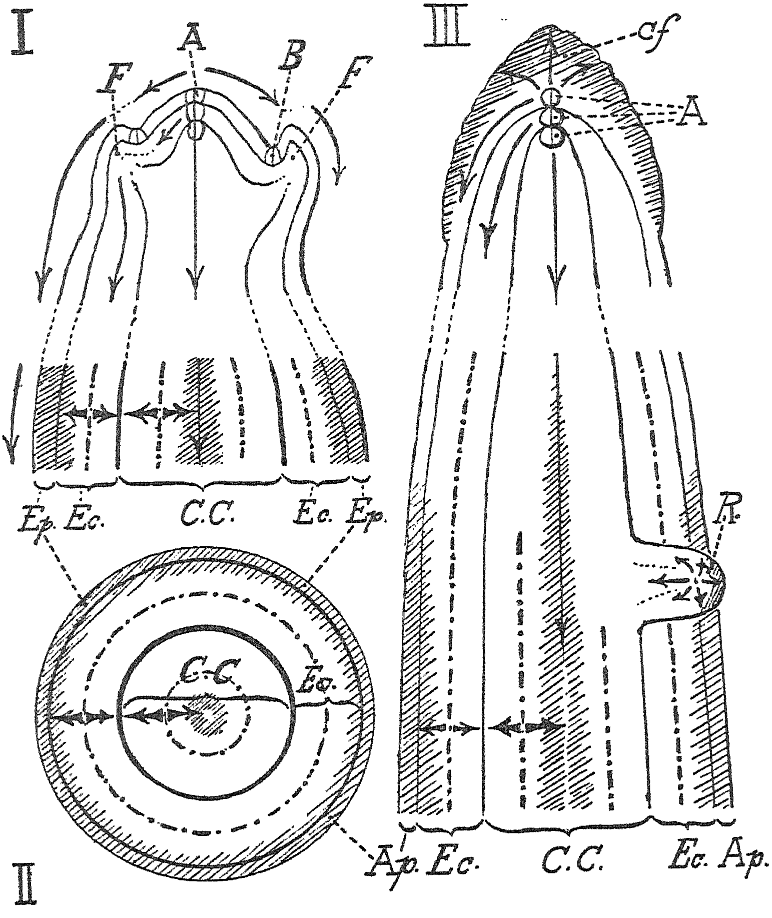


FIG. 7. Polarity of cell senescence in a dicotyledonous plant (74). I: Longitudinal section of a stem. A, initial cells; F, young leaf with its axillary bud B; Ep, epidermis; Ec, cortex; CC, central cylinder. Dot and dash lines indicate cambial layers; hatching, zones where the tissue senescence reaches its maximum. Arrows indicate the directions of increasing senescence of the cells. II: Transverse section of a stem or a root. III: Longitudinal section of a root. Cf, root cap; R, young rootlet, issued by endogenic process; Ap, piliferous layer.

ity would correspond to levels of mean pH, approaching the isoelectric point of protoplasmic proteids; whilst zones of too acid pH would be marked by accelerated senescence of a ligneous or suberous type. In grass epidermis the siliceous and suberous cells, with their accelerated senescence, seem to have a lower pH than the neighboring long cells, the latter keeping their vitality (67).

These senescence gradients are controlled by temperature. Nightingale (61) notes that in apple and peach roots at 32° C. the meristems consist of a number of cells smaller than at the optimum (18° C.), and that differentiation of these cells is more rapid than at the lower temperature. This fact seems to be correlated with scale-rated respiration, with accumulation of CO_2 and with a lowering of pH. The result is an increase of lignified elements and of cork layers.

Mitotic Gradients

Strictly speaking, the mitotic gradient would be expressed by the number of cell divisions occurring in a volume-unit during a time-unit. A satisfactory estimate can be obtained, however, from the number of caryocinetic figures per surface-unit in the diverse parts of an organ's section.

Mitotic gradients show a close linkage to the previously discussed cell-senescence polarity; Fig. 8 is almost a negative of Fig. 7. But it would be a mistake to confuse them. It may happen that a dormant bud does not show any caryocineses; nevertheless its cells do not exhibit any traces of specialization or senility; it is thus necessary to examine them separately.

At the tip of the root (Fig. 8, VI, A) the mitotic gradient is convergent toward the small subterminal group of initial cells, but its slopes are unequal in the different directions. Steep toward the root-cap, whose cells rapidly lose their multiplying ability, this gradient depresses progressively upwards (Fig. 8, VII). The caryocinetic activity resumes only in two levels whose ionic properties have been previously discussed (76), the vascular and the cork cambiums (Fig. 8, C, D), and also in some spots of the pericycle where rootlets arise (Fig. 8, VI, R). The axial mitotic gradient of the root is thus given by the curve aa′ (Fig. 8, VII) with adjuncts c, d and r; and the radial mitotic gradient by Fig. 8, V.

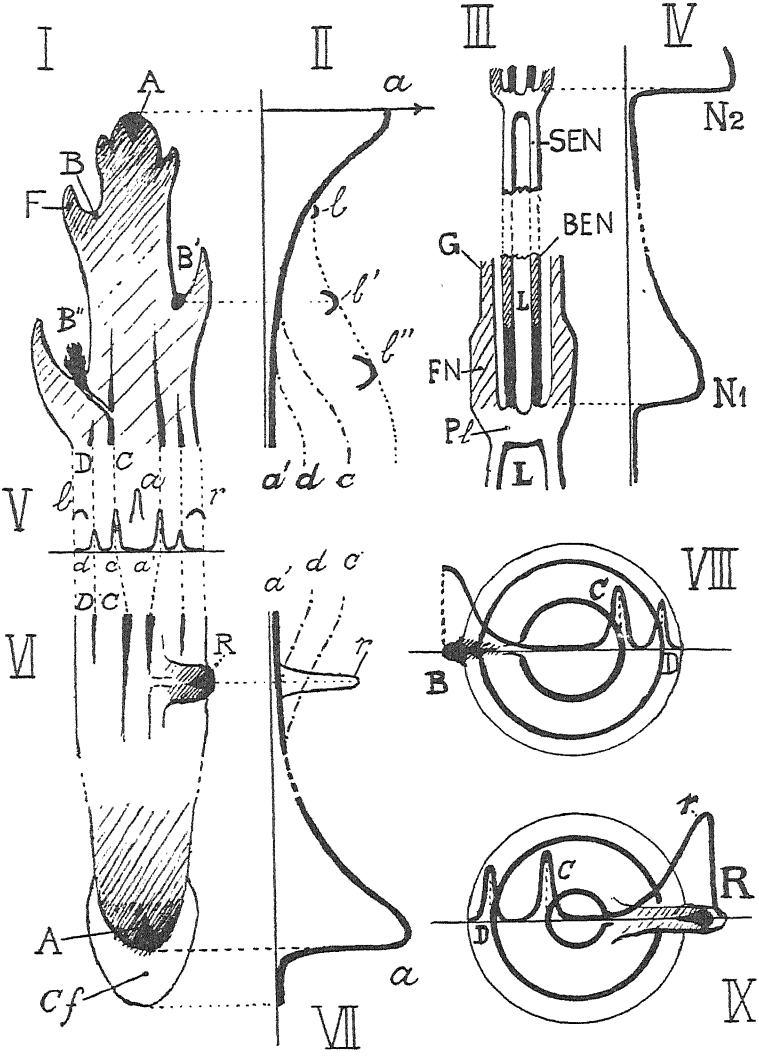


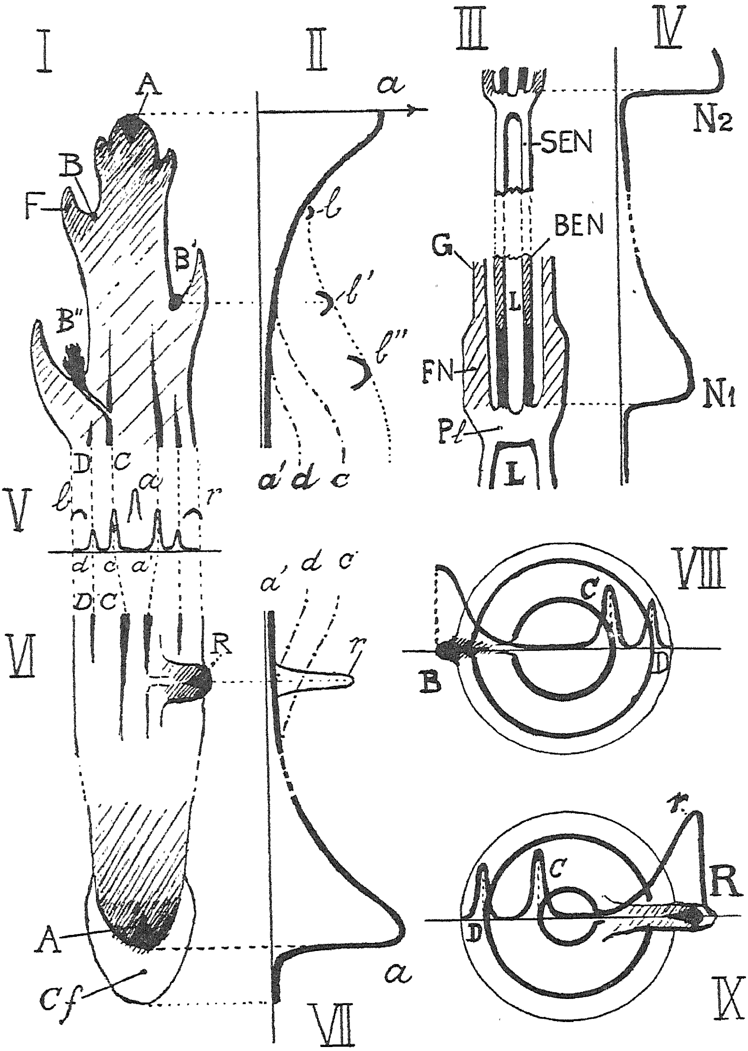
FIG. 8. Mitotic gradients (74). Hatching is darkest where cell division is most active; initial cells and cambial layers in solid black. I: Longitudinal section of a dicotyledonous stem. II: Curves of mitotic densities: aa′, along the axis; b, b′b″, in the buds B, B′ andB″; c, in the vascular cambium C; d, in the cork cambium D. III: Longitudinal section of a grass culm: SEN, summit of an internode; BEN, base of the same; G, leaf sheath; FN, false node (swollen basis of the sheath); Pl, vascular floor of the node; L, central lacuna. IV: Curve of mitotic densities along the internode (longitudinal mitotic gradient) with two maxima corresponding to the nodes N_1 and N_2. V: Radial mitotic gradient. VI: Longitudinal section of a root. VII: Longitudinal mitotic gradient: aa′, along the axis; r, in the rootlet R; c, d, in the cambial layers C and D. VIII: Transverse section of a stem, with curves showing the radial mitotic gradients. IX: Transverse section of a root.

In a stem the morphology of the axial gradient is more complex (Fig. 8, I). It also reaches its maximum in a group of initial cells, here truly terminal, but it shows numerous anomalies, closer to the apex than in the root. In the axil of each foliar appendage a small group of active cells is preserved, forming the initials of a lateral bud. At each of these points the mitotic gradient is so modified as to put itself in convergence toward this secondary focus, a situation that is repeated as often as a leaf is born. Thus the axial mitotic gradient can be shown by the curve aa′ (Fig. 8, II) with the lateral additions bb′b″, concerning the buds, and c and d, regarding the cambial layers. The curve of radial gradient is the same for the stem and the root (Fig. 8, V).

In some cases the axial mitotic gradient of the stem can be still further complicated by the intervention of intercalary growth. For instance, in a grass culm (70) each internode constitutes an independent unit which grows by its base (Fig. 8, III); the axial mitotic gradient is thus multinodal. This structure seems very different from that of an ordinary stem; however, it is possible to conceive the transition from one type to the other. Let us consider an hypertrophy of the secondary mitotic foci constituted by the lateral buds B,B′,B″ (Fig. 8, I), and let us imagine what would happen if these meristematic masses were to invade the entire cross section of the stem; the type of growth of a grass internode would be obtained in this way.

At the same time, by a similar extension of the caryocinetic process, the insertion of the leaf base, primarily crescent-shaped, stretches all around the stem and also acquires a basal growth. Thus appears the well known tubular sheath of the grass leaf. We shall see, in the next paragraph, the importance of this encasing of the internodes by the sheaths to insure the straw verticality. The hypertrophy of this basal proliferation process leads to the formation of the "false node", a thick and rigid cylindric reinforcement at the base of cereal sheaths.

The foregoing facts bring up the difficult question of mitoses determinism and of the motives for its localization. In animal embryogeny numerous workers have shown its connections with the distribution of sulphydriled proteins (17). In plants Hammet and Chapman (39) have recognized in bean roots a high tenure of these substances in the meristems. Auxins, strongly effective on



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The foregoing facts bring up the difficult question of mitoses determinism and of the motives for its localization. In animal embryogeny numerous workers have shown its connections with the distribution of sulphydriled proteins (17). In plants Hammet and Chapman (39) have recognized in bean roots a high tenure of these substances in the meristems. Auxins, strongly effective on cell elongation, can also influence cell multiplication (82, 91). On the other hand, definite hydrionic conditions, as pointed out by J. H. Priestly (76), are required for the preservation of cambial activity. Mitotic gradients appear thus as the tangible expressions of superposed physico-chemical gradations (35, 41, 60, 64, 82, 91).

Maturation Gradients

After the preliminary stages, the cells mature by modifying, both chemically and physically, their contents and membranes. This

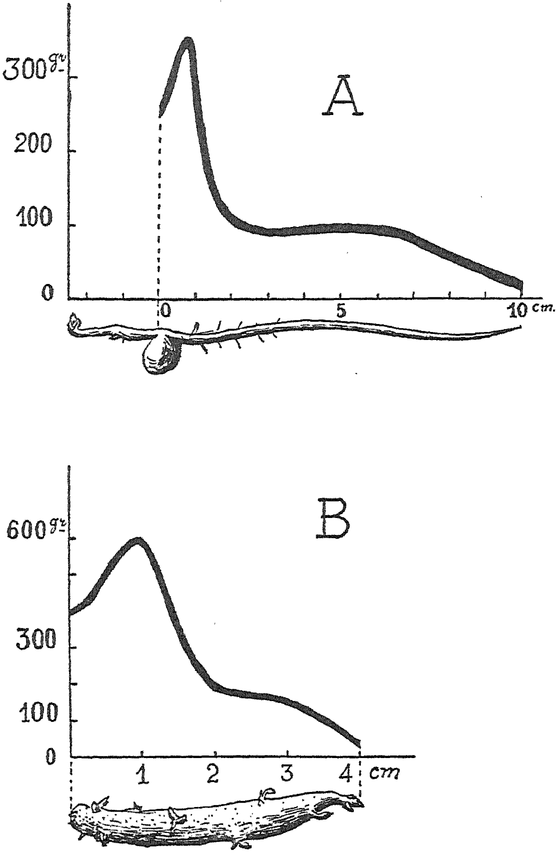

FIG. 9. Gradation of mechanical resistance to sectioning (quantitative expression of maturation). A, in the root of a pea seedling; B, in a potato sprout (74).

fact implies, first of all, a qualitative element, but it is very useful to make it more accurate by numerical measurements. For this purpose I have established a dynamometric apparatus (69,70) permitting measurement of the resistance of plant tissues against mechanical actions, such as cutting, crushing and bending.

1st example. At the root tip of a pea seedling with a root 10 cm. long and a stem 2.5 cm. long (Fig. 9, A), a force of 10 g. applied on the knife is sufficient to cut the organ. Two centimeters higher, the force must be raised to 40 g.; toward the root origin, the required charge is 350 g. The curve transcribing these measurements offers a numerical expression of the maturation gradient (Fig. 9, A). It must be understood that this is only a global indication, the sectioned organ being heterogeneous and its various tissues—wood, parenchyma, *etc.*—possessing different rates of maturation. Also the diameter is not constant, a fact which would compel one to introduce a correction. However, from one place to another, the differences of resistance are so great that these corrections are not sufficient to modify the general shape of the primary curve.

2nd example. On a potato sprout, 4 cm. long (Fig. 9, B), the resistance to cutting is about 20 g. at the tip. Near the basis it reaches 600 g. Here also the meristems are the place of lowest resistance.

3rd example. A more complex case is given by a cereal straw (69, 70). On a growing culm of rye we find at the base of the lowest internode that a force of 1,600 g. is necessary to obtain the section (Fig. 10, E2) ; at the summit of the same, 2,800 g. On the subterminal internode E5 these values become at the base, 40 g.; at the summit, 450 g. The terminal internode E6, just below the spike, offers a still greater range of values: at the base, 20 g.; at the summit, 750 g. This fact indicates that the highest internode, thanks to the immediate influence of the ear, undergoes an accelerated maturation in its upper part. It confirms the conception established, on anatomical grounds, by L. Blaringhem (9) and A. Athanassof (3). These authors have shown that in the wheat culm maturation originates in two regions; first, in the lower internodes; later, in the summit of the highest one. The intermediate part, *i.e.*, the subterminal internode and the base of the terminal one, remains for a longer time in a meristematic state.

This multinodal structure of the maturation gradient could endanger the straw solidity through flexures of the immature bases of internodes. But here intervene the foliar sheaths which, more precociously developed than the internodes, encase them tightly, preventing them from bending. The result is that, to ensure regular growth of the straw without risk of collapse, the elongation and maturation gradients of the sheaths must be rigorously synchronized with those of the internodes. This consistency produces the mode of growth which characterizes the grass family (Fig. 11).

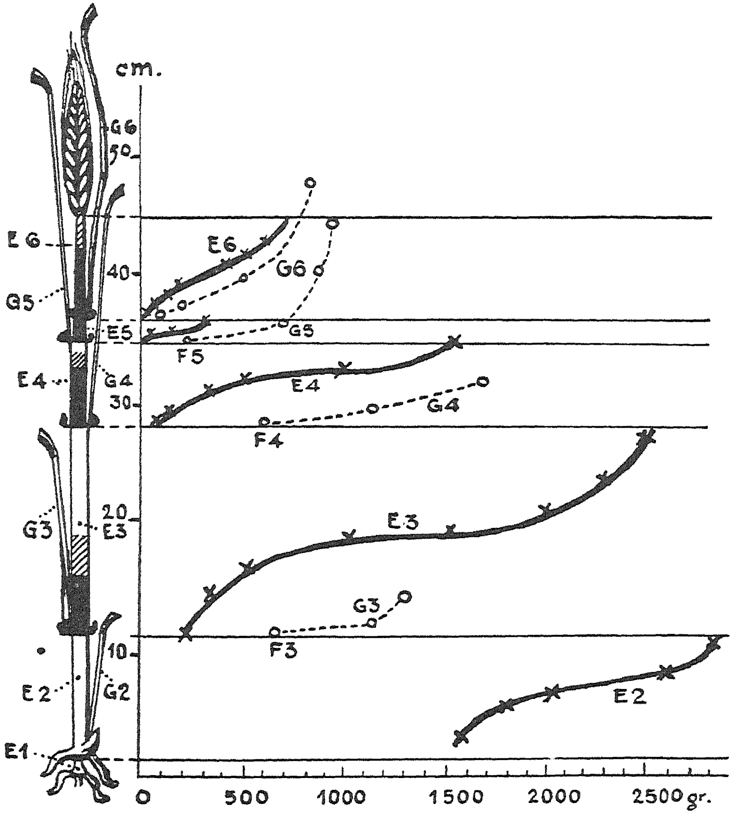

FIG. 10. Gradation of mechanical resistance in a growing rye straw (69, 74). The curves give the resistance to cutting at each point of the internodes (solid lines) and of the leaf-sheath (dotted lines). Young tissues, whose resistance is below 500 g., are figured in black; between 500 and 1000 g., in hatchings. Consolidated tissues, whose resistance is up 1000 g., are white.

Each portion of tissue reaches the open air only when its maturation is sufficient to endure weather inclemencies and mechanical distortions (70, 74).

Such a telescopic mode of growth, which rests on a complex multinodal structure of maturation gradients together with the precise succession of elongation gradients on the time-scale, endows the grass family with a marked advantage in the struggle for life. Instead of being exposed to outward dangers, as would happen if in apical position, the meristems are nested in safety at the segment bases and constantly protected by firm tissues. At worst, when all the upper part of the plant is destroyed, by browsing, fire or anything else, growth can resume in these basal meristems. This peculiarity has bestowed upon the Gramineae a real supremacy over immense regions of the world subjected to cattle grazing and to periodical fires, as on prairies and savannas.

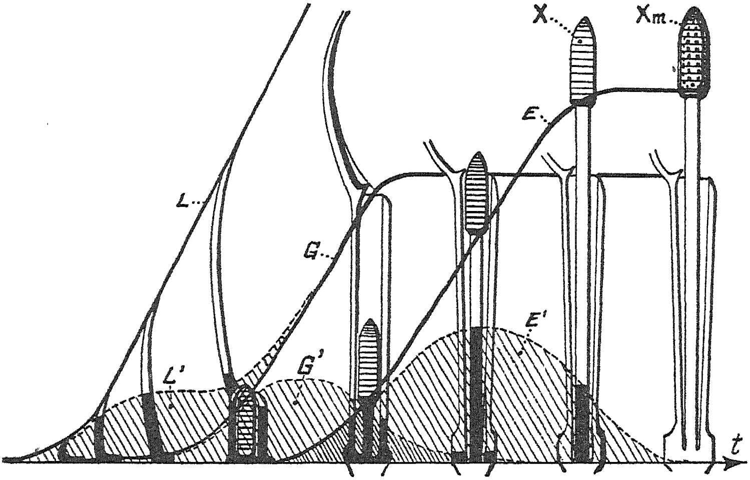

FIG. 11. Scheme showing the growth mechanism of a cereal straw (70, 74). Times are given in abscissae; lengths in ordinates. Solid curves figure the elongation of the blade (L), of the sheath (G) and of the internode (E). Hatched zones L′, G′ and E′ figure the young tissues with a low resistance at the base of the same organs. On each straw these tissues are figured in black. X represents the spike or, more generally, the portion of straw situated above the internode considered. Xm: Mature spike.

In the interest of agricultural practice it may be valuable to pursue detailed studies on growth and maturation gradients of cereals in order to select genetic characters concerning culm rigidity.

This would enable farmers to avert lodging which causes heavy losses annually.

4th example. If we apply the same method of study to a mature straw, some differences become apparent in the features observed during its growth (69). Concerning resistance against cutting forces, the previous sigmoid curve is replaced by one showing a maximum in the middle region of the internode (Fig. 12). Two minima are present near the nodes, which offer again a solid mass.

The resistance against crushing, as shown by the dotted curve in Fig. 12, is almost the reverse of the preceding one. It offers a minimum at half-length of the internode, where the central lacuna is most developed. We find thus a sort of compensation between these two forms of resistance, by which the culm can stay upright in spite of the central hollow. There anew we witness a precise coaptation between the different mechanical gradients.

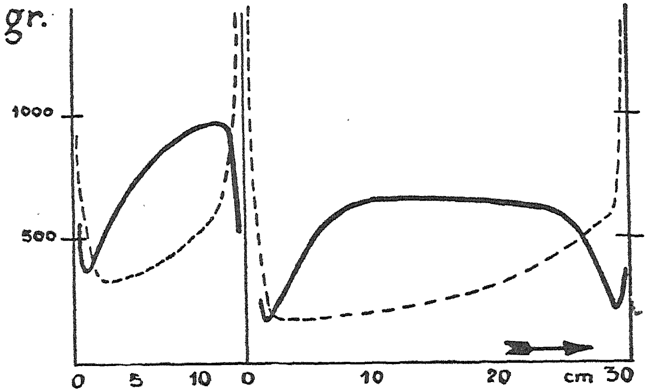

FIG. 12. Gradients of mechanical resistance in a mature cereal straw (69, 74). Resistance to cutting (solid curves) and to crushing (dotted curves) in the two upper internodes of a wheat. (Apex toward the right.)

5th example. All the previous cases have shown us segments of cereal culms growing by their base. In some parts of this family, however, a portion of the stem constitutes an exception to such a rule, for the epicotyl or "mesocotyl[3]" in the tribe Maydeae, in *Avena, etc.,* contrary to the other internodes, grows by its summit (5, 16, 48, 67). This fact can be detected by the classical method of ink marks (Fig. 13, B, C) as well as by observing the mitotic gradient, here ascending, or by measuring mechanical resistance. On an Indian corn epicotyl grown in darkness and 10 cm. long, the force needed to provoke cutting reaches 230 g. at the base and 15 g. at the summit (Fig. 13, A). The curve thus reveals in the epicotyl a basipetal maturation gradient. In the coleoptile P, enclosing the young leaves, resistance increases again from the base upward in conformity with the usual rule.

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[3] The term "mesocotyl" was introduced by Van Tieghem (87, 88), according to an interpretation which was afterwards abandoned (5, 6, 16). Yet, this name is still frequently used.

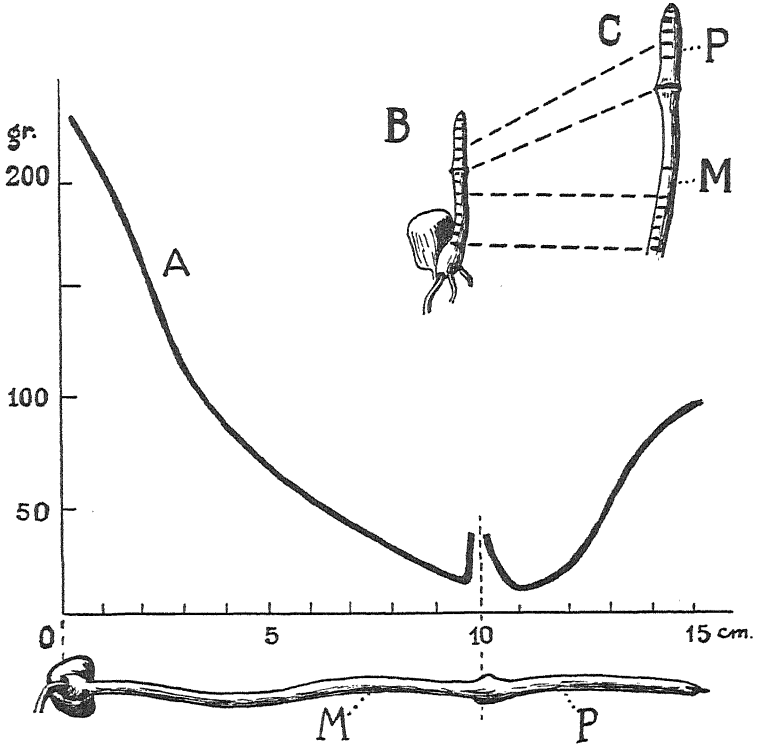

FIG. 13. A: Gradient of resistance to cutting in the epicotyl, M, of an Indian corn seedling (74). P indicates the coleoptile enclosing the young leaves. B: Another maize seedling, bearing China ink marks spaced from 2 m/m. C: The same 16 hours later (30° C., darkness): the epicotyl, M, has grown only by its apical portion; the coleoptile, P, has grown by its base.

Thus, upon crossing the first node of a Maydeae seedling, we witness the complete reversion of all the growth gradients: of mitotic activity, of maturation, of mechanical resistance, of elongation.

Alone of its kind, the first node remains locked up between two meristematic masses.

This mechanism implies an ecological advantage for Indian corn, inasmuch as it insures the rooting of its coleoptilar node near the soil surface, sunlight checking the growth of the unprotected meristem of the epicotyl when it reaches the open air. Thus a slight pecularity of the growth-gradients can play a considerable role in the life-efficiency of a species.

Elongation Gradients and Biometric Gradations

Plant organogenesis depends primarily upon localization of cell-division foci, upon polarity of cell-maturation and on orientation and rate of cell increase. Having examined the first two points we can now approach the third.

From the base to the summit of a plant the dimensions of homologous parts—cells, groups of cells, anatomical units—show progressive variations. We find here a static transcription of the dynamic fact constituted by the auxetic gradients and their physiological commands.

All measurable elements can be included among biometric gradations. We shall choose examples in the elongation of internodes in the Gramineae. Novacki has given a formula expressing their increase from the base toward the summit of a culm (3): "The length of an internode is the average between the length of the two neighboring internodes", and F. E. Lloyd (50) another: "The terminal internode of the wheat is equal to half of the entire culm and to twice the length of the subterminal internode". It is possible to obtain a more accurate formula (68–70). Let us put in abscissae the logarithms of the rank of the internodes from the straw base, and, in ordinates, the logarithms of their lengths. Frequently the dots tend to be on a straight line (Fig. 14, I). If we establish the curve on the average of many culms we observe that, the more these latter are numerous, the straighter the dots are aligned. The lengths of successive internodes are thus given by the formula:

$$\log Y = K \cdot \log N + \log A$$

whence:

$$Y = A \cdot N^k$$

where Y is the length of an internode; N, its rank; A, a constant; K, a coefficient expressing the slope of the line and, thus, the biometric gradient.

The value of *K*, as well as the shape of the curve and its irregularities, depend, on one hand, upon the species or variety; on the other hand, upon the circumstances of growth, chiefly of flowering. The more the blooming is accelerated, the stronger the gradation is. In *Mibora verna*, a small annual herb with a very short life-span, this gradation is brought to an extreme; for instance, the lengths of successive internodes, in millimeters, are: 0.4, 0.7, 1.6, 6, 100 (Fig. 14, curve II). We see that, even in logarithmic co-ordinates, the dots are on a concave line. In this case the previously given formula does not fit; the rapid evolution of flowers provokes such an hypertrophy of the uppermost levels that it would be necessary to use a more rapidly progressive exponential formula.

On the contrary, when a culm is sterile the gradation disappears; all the internodes remain about of the same length or even decrease toward the apex by exhaustion of the shoot vitality. For instance (70), on the culm of a bamboo (*Phyllostachys*) they present, first, an increasing length, up to a maximum of 23 cm. Then they decrease, measuring, in centimeters, 23, 22, 18, 18, 17, 17, 17, 15, 15, 15, 13, 12, 11, 10, 9, 8, 6.5, 5.5, 4.2, 3.4 and 2.4 cm.

Progressive gradation is absent because the stem apex is not sexualized. The flowers will appear, after many years, only on lateral branches; thus they are unable to influence in any way the building of the principal culm. But we can observe that, thanks to their action, the progressive gradation of internodes reappears on the floriferous lateral off-shoots.

In herbaceous species also we can observe sterile culms. For instance, in *Holcus lanatus* we find together flowering straws, offering a normal progressive gradation (Fig. 14, A in III), and straws without any flowers. On one of the latter the size of successive internodes in millimeters will be: 27, 33, 40, 33, 36, 38, 37, 32, 37, 34, 33, 20 cm. (Fig. 14, B in III). There no gradation is noticeable, but only an irregular fluctuation reaching 20% (70).

These observations put in full evidence the influence exerted on stem elongation by the maturing flowers. Experimentation confirms this view, for in growing culms of wheat, a resection of the young spike stops the elongation of terminal internodes (70). However, one cannot allege that the operation has injured the meristems, these being located at the base of the organ and untouched. Of course it is easy to verify their integrity, for if a

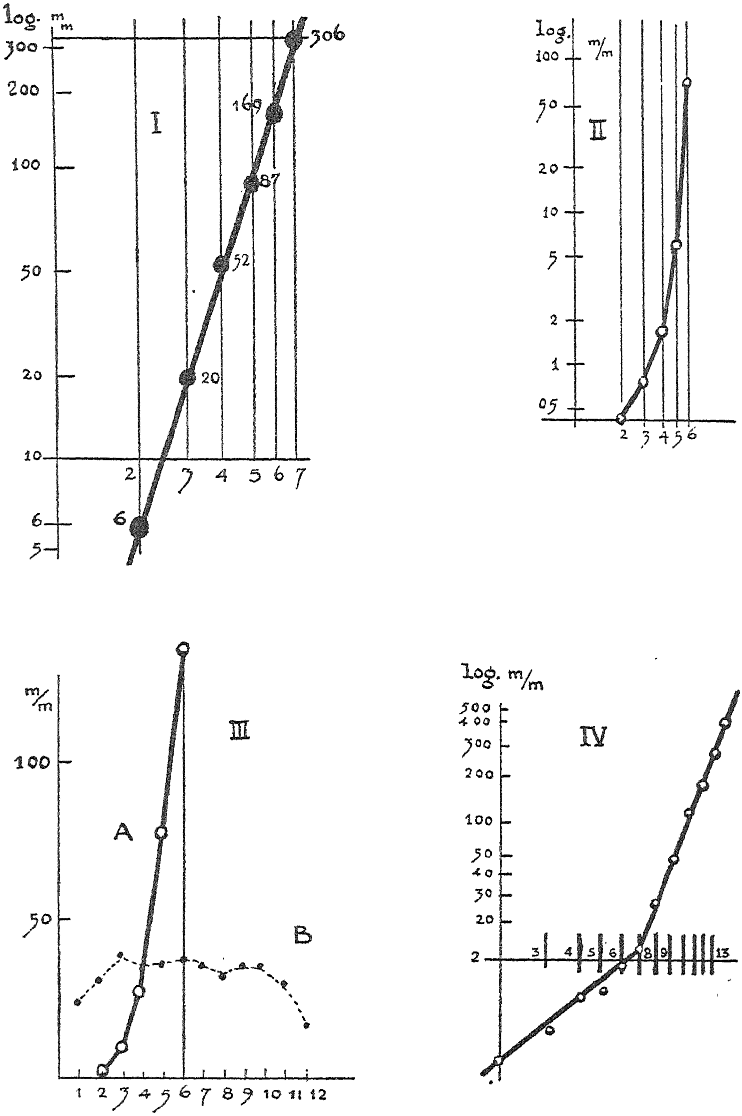

FIG. 14. Biometric gradation in straws. Ordinates represent lengths of internodes; abscissae, order number of these internodes from below upward. I: *Triticum monococcum* (average of 20 straws) in logarithmic co-ordinates (70,74). II: *Mibora verna,* id. III: Holcus lanatus (ordinary co-ordinates): A, fertile stem; B, sterile stem. IV: *Oryza sativa* var. "Lua-Voi", logarithmic co-ordinates (28).

cut-off spike is replaced on the decapitated internode and maintained in place by a small block of agar, growth resumes at once.

These facts are obviously due to the influence of auxins emitted by the flowering culm's apex. And, what is most interesting, they show the variations occurring in the emission of these growth substances. From one internode to the other the observed gradation is due to an exaggeration, not only of cell enlargement (hypertrophy) but also of cell multiplication (hyperplasia). This observation can be compared with those concerning the effects of external factors. When an organ is checked in its growth by light, both cell number and cell length decrease (6).

It can be noticed that the formula giving the relative length of internodes, $Y = A \cdot N^k$, is analogous to the classical allometric growth formula established by J. S. Huxley (44, 45, 47). The difference is that a rank number, *i.e.*, an arithmetic progression of ratio one, takes the place of the reference length. We can conceive the progressive gradation of the culm as resulting from the superposition of two orders of phenomena:

a). First occurs the building of the vegetative axis. This begins in the bud by the creation of a pile of flat discoid units and continues by their stretching out, one after the other, from the base upwards. This phenomenon is similar to those offered by the animal kingdom in the strobilization of the scyphystom larvae of jellyfishes; in the formation of the segments of a worm; in the metamerization of a vertebrate embryo, *etc.* It leads to the creation of a segmentary structure showing the interplay of axial multinodal gradients. But the pattern of growth is ruled here by the successive dominance of one meristem upon all the others with regular transmission of this supremacy from the base upwards. The term "plastochron" has been coined (1, 30) to designate the period of time elapsing between the production of those successive segments by the apical meristem. This notion permits us to describe accurately the phases of elaboration of the terminal strobile.

In a sterile culm all these segments acquire about the same final size, the apical hormones being emitted at a constant rate. There intervenes only the first order of phenomena, vegetative metamerisation. In our formula, $Y = An^k$, we have in this case: $K = 0$, and in consequence: $Y = A =$ a constant.

b). When the culm is floriferous, phenomena of a second order interfere with the preceding ones. During the course of its growth this segmentary structure is modified, stimulated by the sexualized apex. Thus another gradient of elongation—this time uninodal and of sexual obedience—is superposed on the previous vegetative gradient of multinodal shape.

In our formula this second gradient manifests itself by the appearance of the coefficient K. The more rapid the increase of hormone production by the apex, the greater becomes K, and the steeper the slope of the figurative line. In *Mibora* flowering is so accelerated that the second gradient overcomes the first. The number of internodes is reduced and the terminal one becomes hypertropied, while the lower ones remain very short (Fig. 14, II).

On the contrary, when flowering intervenes late, the first phenomenon can develop freely. The number of internodes produced is high, while the gradation remains moderate; coefficient K is then low. External circumstances can modify this structure. In the same variety of wheat, for instance, individuals sown during Spring show a stronger gradation than those sown during Fall. Greater rapidity of floral evolution produces in the former an exaggeration of the elongation gradient (70).

One of my students, Duong Huu Thoi (28), has shown that on a rice culm it is possible to observe different rates of gradation corresponding to successive phases of development (Fig. 14, IV). In the first, purely vegetative, corresponding to the vertical rhizome, the gradation is feebly marked, with conspicuous fluctuations. In the latter, corresponding to the erection of the floriferous culm, the gradation suddenly becomes very strong because of the intervention of the blossoms and of the increased hormone secretion they provoke.

To each of these phases taken in particular, corresponds a formula of the $Y = A \cdot N^k$ type, but with coefficients A and K showing different values, K being much greater at the final phase.

On Indian corn embryos separated from their endosperm, Andronescu (2) has reported that growth is slower and internodes less numerous than on normal ones. The lack of the proteins, carbohydrates and growth substances contained in the endosperm seems to check the primary process building the multinodal gradients outlining the internodes. Very likely reception of auxins is not the only factor in all these cases of differential growth; the entire set of physico-chemical gradations also intervenes. Priestley (76, 77) has pointed out the importance of low pH for cell elongation. I have observed (70) that sectioned internodes of cereals, placed in slightly acid solutions, can resume growth as if they had received their normal distribution of auxins[4]. Thus different factors can, to a certain extent, compensate one another to insure elongation.

Culpepper and Moon (22) have studied the transformation of physico-chemical gradients during the development of *Asparagus* stems and their relations with growth. They observe that the highest moisture is located in a definite region "between that at which growth is maximum and the point when it ceases"; also that the total sugars are most concentrated at the stem's base, decreasing rapidly in the growth zone. Conversely nitrogen is lowest at the base and increases near the tip. Hicks (42) notes also, on willow cuttings, that shoots develop in areas of lowest C/N ratio, and roots in those of highest. Thus appears once more the close relationship between growth and chemical gradients (41, 64), trophic as well as hormonal, hydric as well as redox or ionic.

SUMMARY

The concept of gradients, currently used in physics, has numerous applications in the study of plant organogenesis. The smallest portion of plant tissue, the most reduced group of cells, reveals the presence of various kinds of gradations.

A classification of the different types of gradients which occur in birth and functions of the plant body has first been established. They are in constant interaction. The physiological gradients, concerning intensity of respiration, absorption, photosynthesis, *etc.*, induce the appearance of various kinds of physico-chemical gradients: pH, rH, concentration of carbohydrates, *etc.* In turn, the latter provoke local modifications in the evolution of the cells, thence the histological and anatomical gradations easy to observe in the structure of organs. Finally these histological differentiations determine the functions of the maturing tissues, and the circle is closed, all the gradients being elements of a coherent system whose parts are all interdependent.

Series of facts have been grouped concerning the gradients of pigmentation, moisture, resistance to heat and to parasitical organ-

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[4] An activation of pro-auxins by low pH may intervene in this case.

isms. Then the organization of gradients has been described in a growing plant: gradients of cell senescence, mitotic activity, elongation, maturation and mechanical resistance. A consideration of these phenomena permits an explanation of the growth mechanism peculiar to the grass family. It consists of the progressive telescopic extension of parts growing by their base and sheltered by the rigid sheaths of the leaves.

The intervention of sexual activity deeply modifies the gradient system of a plant. This fact is apparent in many ways; in the coloration of the upper leaves, in their morphogenesis, in the distribution of essential oils, *etc.* It may be seen also in the increasing length of internodes of a grass culm. Thus we observe the interference of two series of phenomena. The first, being rather slow and developing at a constant rate, is the edification of numerous segments, all potentially similar; the second, sudden and operating with an increasing rate, is the sexual crisis.

In the second part of this review, to be published later, we shall find in histological gradations other manifestations of the floral influence. In that account we shall discuss the mode of evolution of the cells and the progressive expression of cell potentialities. Finally the modifications of the gradients under the influence of external factors—physical, chemical, biotic—will be considered.

To complete this first section of a very extensive subject we shall now give only a partial bibliography. A complementary bibliography will be given with the second section.

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DESMIDS

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INTRODUCTION

Since their discovery in the early days of microscopy and following the establishment of the first genus (*Closterium* Nitzsch)[1] in 1817, the bizarre desmids have attracted much attention. Although minute in size, their beauty of form and endless variety of shapes are equalled by many unique biological characteristics. Hence they have long been favorite subjects of exploration by both amateur and professional microscopists, and continue to be an intriguing group of organisms in biological research.

The common name, desmids, refers to the fact that many occur in 'chains' (filaments), although the vast majority exist as solitary cells. Sometimes, however, they are in colonial masses held in copious mucilage secretions characteristic of desmids. Furthermore, as outlined below, the common name has come to be used for plants actually distributed among three different families, according to some students, in the order Zygnematales (Conjugatae) of the Chlorophyta. Their present taxonomic status, at least temporarily well established, has been arrived at by much shifting of thought and opinion, and they have held a variety of positions in plant classification. At one time desmids, as well as many other kinds of algae, were relegated to the animal kingdom (Ehrenberg, *e.g.*). When they finally became included among the algae they were variously treated, sometimes associated with diatoms to form the Zygophyceae, sometimes regarded, together with other Zygnematales, as a group separate from the Chlorophyta (Conjugatae, 53, 76).

A SYNOPSIS OF THE ZYGNEMATALES (CONJUGATAE)

Zygnemataceae

1. *Mougeotia* Agardh. 1824.
2. *Debarya* Wittrock. 1872.
3. *Mougeotiopsis* Palla. 1894.
4. *Zygnema* Agardh. 1824.
5. *Hallasia* Kolderup-Rosenvinge. 1924.
6. *Zygogonium* Kuetzing. 1843.
7. *Pleurodiscus* Lagerheim. 1895.
8. *Entransia* Hughes. 1943.
9. *Spirogyra* Link. 1820.
10. *Temnogametum* West & West. 1897.
11. *Sirogonium* Kuetzing. 1843.

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[1] *Echinella* (untenable) was described by Acharius in 1810. Beiträge zur Naturkunde 2: 340–342. 1810.

Gonatozygaceae

1. *Gonatozygon* De Bary. 1856.
2. *Genicularia* De Bary. 1858.

Mesotaeniaceae (Saccoderm Desmids)

1. *Mesotaenium* Naegeli. 1849.
2. *Cylindrocystis* Meneghini. 1838.
3. *Netrium* Naegeli. 1849.
4. *Roya* West & West. 1896.
5. *Spirotaenia* De Brébisson. 1848.
6. *Ancylonema* Berggren. 1870.

Desmidiaceae (Placoderm Desmids)

1. *Closterium* Nitzsch. 1817.
2. *Spinoclosterium* Bernard. 1919.[2]
3. *Penium* De Brébisson. 1844.
4. *Pleurotaenium* Naegeli. 1849.
5. *Ichthyocercus* West. 1897.
6. *Docidium* De Brébisson. 1844.
7. *Triploceras* Bailey. 1851.
8. *Tetmemorus* Ralfs. 1844.
9. *Euastrum* Ehrenberg. 1832.
10. *Euastridium* West & West. 1907.
11. *Cosmarium* Corda. 1834.
12. *Cosmocladium* De Brébisson. 1856.
13. *Oocardium* Naegeli. 1849.
14. *Arthrodesmus* Ehrenberg. 1838.
15. *Spinocosmarium* Prescott & Scott. 1942.
16. *Xanthidium* Ehrenberg. 1837.
17. *Micrasterias* Agardh. 1827.
18. *Onychonema* Wallich. 1860.
19. *Sphaerozosma* Corda. 1834.
20. *Spondylosium* De Brébisson. 1844.
21. *Hyalotheca* Ehrenberg. 1841.
22. *Phymatodocis* Nordstedt. 1877.
23. *Desmidium* Agardh. 1825.
24. *Gymnozyga* Ehrenberg. 1841.

At present the so-called desmids fall mostly into two major groups, Saccoderm (Mesotaeniaceae) and Placoderm (Desmidiaceae), whereas two genera (*Genicularia, Gonatozygon*), commonly thought of as desmids and often occurring with them in nature, constitute the Gonatozygaceae (17). The total composition of these three families of the Zygnematales involves about 4000 species[3]. Of these, 41 belong to the Mesotaeniaceae; about 20 to the Gonatozygaceae; and the majority, about 3900 species, make up the Desmidiaceae[4]. The numerous geometric forms and the variety of patterns in wall decoration of these thousands of species, in addition to the varied shapes of chloroplasts, explain the aesthetic interest in desmids.

Quite aside from this, desmids have invited investigation because of a number of problems which they pose. Among these the ever present question of their phylogeny, as well as evolution within the group, have merited considerable thought and speculation. This problem is especially important inasmuch as their history, or at least a concept of their evolution, has an important bearing on notions regarding the development of the entire order Zygnematales and its place within the Chlorophyta. Again, unique features found in the mode of cell division, methods of sexual reproduction, and their wall structures are examples of phenomena which come up for repeated investigation. Notwithstanding the enormous amount of research already applied they still present many biological problems worthy of serious attention. Sampaio has evidence from his culture studies that bacteriocidal action is produced by *Closterium acerosum* comparable to that of penicillin. Distribution of desmids in time and space provide the ecologist with ample material for investigation. As a class they exhibit a high degree of physiological selectivity of environment, apparently being very sensitive to water chemistry; and they are so abundant in and so characteristic of some situations that reference is often made to "desmid habitats".

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[2] Some students regard this genus as synonymous with *Closterium*.

[3] The published total number of species of the Chlorophyta (5,500) is obviously incorrect.

[4] See synopsis of the Zygnematales, above.

CELL MORPHOLOGY

The two primary classes of desmids, Saccoderm and Placoderm, are separated principally by a fundamental difference in wall structure. The former (Mesotaeniaceae) have membranes composed of a single piece (*Netrium, Cylindrocystis, e.g.*) and the cells are not constricted in the midregion. The Placoderm (Desmidiaceae) or true desmids (*Cosmarium, Micrasterias, e.g.*), on the other hand, possess a wall composed of two closely but slightly overlapping sections which fit together in the midregion of the cell where, in most genera, it is more or less deeply constricted. Thus the typical desmid cell consists of two symmetrical halves (semicells) which are mirror images of each other, connected by a narrow isthmus. The median incision, called the sinus, may be narrow (linear) and closed, or it may be open, that is, with margins diverging outwardly from an acute or rounded inner apex. The early students regarded constricted desmids as being composed of two cells when the sinus was very deep and the isthmus correspondingly very narrow and not altogether obvious when viewed under low magnification. Because of the peculiar type of cell division in desmids, described below, one semicell is always older than the other unless the cell is an individual recently developed from a zygospore and has not yet divided. It is interesting to speculate on just how old one semicell of a desmid may be, having come down through many generations by cell division.

Although symmetrical in front, top or side view, the geometric figure changes accordingly, and the same individual presents an entirely different aspect when seen from various angles, thus adding to their aesthetic qualities. Because of the variation in symmetry in different views it is necessary to see the cell from the front, top and side for taxonomic considerations. Two or more species may appear similar and almost indistinguishable in front view (designated in desmid illustrations as 'a'), but quite different when seen from the top ('b'), or the side ('c'). Although the front view of a desmid species may vary in outline and proportions, the vertical view conforms more constantly to a specific shape.

Whereas the cell wall of the Saccoderm desmids (*Netrium, Cylindrocystis*) is simple, practically structureless, and is composed of but a single layer, the Placoderm desmids and the Zygnemoideae (including *Genicularia* and *Gonatozygon*) have walls of three layers, the middle one of which in the former may be highly decorated. The true desmids have an inner firm layer of cellulose and externally a layer of cellulose impregnated with pectic material and, in some, iron compounds. In certain species of *Cosmarium,* but especially in *Closterium* and *Penium,* the iron may be so abundant as to tint the wall yellow to rusty brown. Because of the difference in their age often a great contrast in color of the two semicells is shown. The iron (iron salts) may be evenly deposited or in pattern-like bands, especially near the isthmus. Höfler (20) is of the opinion that the iron is in definite chemical unity with the wall material and not merely adsorbed. Outside the cellulose layer is a film of mucilage which gives most desmids a slippery texture, characteristic of Zygnematales in general.

The wall of Placoderm desmids is further differentiated from the Saccoderm by the possession of mucilage pores, often very prominent and sometimes pattern-like in their arrangement (*Euastrum Jenneri, e.g.*), whereas in many instances the pores are minute, numerous and more or less evenly distributed over the wall. In *Euastrum pingue,* for example, there may be two large pores, one in each face of both semicells characteristically and specifically excentric. It is through the secreting action of pores that the mucilaginous layer and characteristic sheath are maintained. In the case of larger pores there is a narrow canal from a swelling within the inner membrane. Outwardly it enlarges into a collar which Lütkemuller (32) calls a "pore organ". Within the gelatinous investment of the desmid cell, very wide at times, there frequently appear fibrils of mucilage radiating out dirctly opposite the mouths of the pores (*Cosmarium, Staurastrum, Hyalotheca*).

The mucilage sheath enters into the biology of the desmid cell in several ways. In *Onychonema* spp., for example, the mucilage is tough and rubbery, forming intercalary pads about the end walls that hold the cells in filamentous arrangement. The mucilage may be firm, forming a solid case about the cell (*Euastrum pinnatum, e.g.*), apparently serving as a protective sheath in which the cell exists in a dormant condition; this has not, however, been demonstrated in culture. Whether this is a true "akinete" has not been determined, but it seems likely that desmids pass unfavorable environmental conditions in such a manner, supplementing the zygospore stage of their life history. The mucilaginous sheath is especially abundant about such colonial forms as *Cosmocladium* in which a number of *Cosmarium*-like cells are inclosed in a colorless sphere and often tied together by pairs of parallel interconnecting fibrils. Sometimes the outer mucilage layer is thin and close, but more often there is a prominent envelope, as in the filamentous *Hyalotheca mucosa, Desmidium* spp. and some forms of *Staurastrum* and *Cosmarium.* The mucilaginous sheath can be demonstrated clearly by mounting living cells in india ink diluted with ammonia.

Rather curiously the mucilage of some desmids becomes the stratum for the attachment of various epiphytic organisms, some of which seem to be specific (*Peroniella hyalothecae, Stipitococcus capense*). In general, however, the slimy outer layer of all Zygnematales seems to be resistant to the attachment of epiphytes, and they are seldom, if ever, overgrown with other organisms as are many other algae in the same habitat. One might suppose that the mucilaginous sheath would be a favorable medium for bacteria, but the author has never seen microorganisms in this association, except for apparent incidental adherence, West's (68) statement to the contrary. Because of sticky mucilage desmids commonly are found attached to submerged stems and petioles of aquatic plants (*Utricularia, Chamaedaphne*). In the back waters and littoral zones of the Panama Canal and along the Chagres River the author found a veritable "zoogloea" of desmids, forming very extensive films over submerged vegetation. In the rare genus *Oocardium,* small ovoid, slightly constricted cells are encased in the ends of parallel mucilaginous tubes which are embedded in lime. An association of these plants forms a hemispherical incrustation on aquatic mosses and on stones, especially in hard water. The well known movement exhibited by some desmids (*Closterium,* especially the tumid crescent-shaped species) is achieved by the secretion of mucilage against a substratum (16). Thus if a collection of living material is allowed to stand for a time in a glass receptacle the desmids will become concentrated on the illuminated side after a few hours, the response to light somehow influencing the direction of movement. As demonstrated long ago by Stahl (59, 60), *Closterium* achieves locomotion by swinging through an arc of 180 degrees, attaching itself first at one pole and then at the other. When given an opportunity some desmids show an interesting polarity, taking a position so that the younger semicell is nearest the source of illumination. The presence of desmids in a favorable habitat can be detected easily by the slippery quality which they impart to *Sphagnum, Utricularia* and other substrates in acid situations.

In addition to mucilage pores the structure of Placoderm desmid walls may be complicated further by punctations, scrobiculations, granules, verrucae and spines. These, sometimes in combination, together with tubercles, protuberances and arm-like extensions, provide ornate patterns of specific design and contribute much to their beauty and interesting variety. The symmetry of cell outline and of wall decoration is reflected in the symmetry of the protoplast. Even among the Saccoderm desmids, in which there is no constriction to form semicells, many show internal duplex organization. The visible evidence of this symmetry is the position and arrangement of chloroplasts, large and few in number, as is characteristic of all Zygnematales. The chloroplasts are usually dense green in color and vary much in shape. There may be spiral ribbons (*Spirotaenia*), star-shaped masses (*Penium*), geminate stars (*Cylindrocystis*), axial cones with radiating plates (*Closterium*), parietal ribbons (*Pleurotaenium*), thin plates (*Roya*), axial masses with radiating arms (*Cosmarium*). The number of chloroplasts is usually specific, and in some cases is helpful in making taxonomic determinations. Generally speaking there is a single chloroplast in each semicell in the Placoderm desmids, but there may be two each (rarely more), whereas in a few small cells and especially in the Saccoderm desmids there is but one chloroplast (*Spirotenia, Roya, Mesotaenium*). In keeping with the general rule among the Zygnematales chloroplasts are axial, but a parietal position is taken in some (*Pleurotaenium, Xanthidium,* and a few species of *Cosmarium*), especially in those species which have two or more chloroplasts in each semicell. The parietal position is regarded as indicating a more advanced evolutionary development. An apparent parietal location may result from the fact that the axial core (*Closterium, Netrium,* and some forms of *Cosmarium*) have radiating ridges which flatten themselves and become lacinate bands against the wall. In *Pleurotaenium* the chloroplast is at first axial but later breaks into ribbons which become parietal. There may be anastomosing of the lobes of these bands so that very elaborate structures result in that genus and in *Closterium*.

An invariable feature of desmid chloroplasts is the pyrenoid. These starch-accumulating bodies, large in all Zygnematales, are usually buried in the axial core, although Carter (7) found them occasionally free in the cytoplasm, and they may occur one in each chloroplast, or vary in number in certain species. In *Closterium, e.g.,* there is often an axial row, but in some species of the same genus they are scattered. It has been shown that pyrenoids may divide during cell division, although they may develop as new structures as in many other algae. Carter (7) and Ducellier (12) have studied the chloroplast and pyrenoid relationship cytologically. The former author called attention to fibrillar extensions of the chloroplast which approach the nucleus in such a way as to suggest an organic relationship.

Desmids are of course uninucleate. The nucleus, cradled in a web-like mass of cytoplasm, is centrally located in the midregion between the chloroplasts of the two semicells, or in the isthmus. In *Genicularia,* however, the nucleus is lateral to the chloroplast midway in the cell. Cyclosis of the cytoplasm is often clearly visible because of the numerous and conspicuous granules of ergastic substances and crystals of waste material, notwithstanding the large and dense chloroplasts which often nearly fill the cell and so mask the cytoplasm.

Another interesting feature of some Placoderm desmids are vacuoles containing actively vibrating granules. These vacuoles occasionally appear throughout the cytoplasm in forms which have parietal chloroplasts, as in *Pleurotaenium,* whereas in *Closterium* they may occur in the troughs between the longitudinal radiating plates, and between the chloroplasts in the semicells of *Cosmarium.* In addition there are prominent terminal vacuoles in *Closterium* and *Pleurotaenium.* According to Fischer (14) the Italian, Lanzi, thought that malaria was caused by the granules in these vacuoles which were released by death of the algae in the Pontifical ponds.

The vacuolar system has been the subject of much study (14, 15, 27, 29, 54, 61, 82), but its full significance and possible rôle are still not clearly understood. At least some of these irregularly-shaped granules are known to be gypsum, thought to be organic by Naegeli, and Steinecke (61) has advanced the idea that they function as statoliths inasmuch as they are often found lying in the lower part of the vacuole. This view has not been generally accepted, however, and their behavior presents evidence to the contrary. Fischer (14), in a detailed study of crystals in several genera, found them to occur in the cytoplasm as well as in vacuoles, and reports a zygospore (*Closterium*) with crystals which were undissolved after treatment with sulphuric acid. This author believed that, in addition to Brownian movement exhibited by the granules, they have a secondary motion caused by pressure changes in the cytoplasm. He claims further that the cavities occupied by the granules are not true vacuoles but merely spaces within the cell sap determined by the shape of the chloroplast (*Closterium*) as that structure develops and delimits the polar cytoplasm. He points out that if growth of the chloroplast in *Closterium* ceases early a large space is left in which but a few crystals move about freely. The crystals, he found, are formed in the cytoplasm, are picked up by the circulating stream, carried forward to the poles where some, by chance, collect in the terminal pocket and are put out of circulation. Here they may be held in a whirlpool action and do not necessarily sink to the bottom of the cavity which they would do if only Brownian movement were responsible for their vibration. In small species with long drawn out points and short chloroplasts the vacuoles are likewise long and conform to the shape of the cell.

Frey (15), applying Stokes law, determined that desmid crystals have a density 2–2.5 times that of water. He is in agreement with many other observers in the belief that granules are excretory products, and he goes so far as to compare vacuoles with the excretory organs of protozoa.

In reporting on his studies of desmid granules, Fischer also discusses "little *Zygnema* balls", granules which appear in great number when plants are aging. He draws some analogy between these and crystals in the desmids and expresses the opinion that these are waste products and that their accumulation in large numbers spells death for the cell unless cell division occurs. In his study of ten desmid genera he divides them into three groups: those in which crystals are always present, those in which crystals appear only in unhealthy or dying cells (*Cosmarium, Micrasterias, Euastrum*) and those in which crystals do not appear. Fischer calls attention to the fact that desmid zygospores have crystals only for those species which exhibit them in the vegetative condition.

Kopetzky-Rechtperg (27), in his exhaustive study of crystals, tested their solubility with various chemicals and observed their movements. He denied a statolithic function, suggested by Lotsy, but suggested interestingly enough that they induce thigmotropic response by their position in the bottom of the cavity where they cause a greater extrusion of mucilage on the substratum. If they have a statolithic function, he points out, it is only incidental and is not to be thought of as being comparable to the phenomenon observable in cells of some higher plants. He considered the granules to be only waste products of metabolism which become evenly distributed in the two semicells.

Whereas in a few species there seems to be a constancy of number of granules in the terminal vacuoles, or a constant range of numbers, this does not hold true for most (82). Kopetzky-Rechtperg believes that the number of crystals is incidental to varying factors and is not constant for a species, but that the shape of the crystals is specific for many forms which have been studied. Wurtz (82) has studied the curious phenomena attending the behavior of gypsum granules in *Closterium Dianae*. The vacuoles, spherical in some species, were demonstrated by vital staining to be irregularly conical or elongate, tapering toward the apex of the cell. Around the basal part of the vacuole there is an active, undulating cyclosis of the protoplasm. The lower portion of the vacuole retains its form, more or less, but in the upper part, because of osmotic pressure changes in the unstable cytoplasm, the vacuole continually undergoes distortion and fragments into small vacuoles which then coalesce with the large one again, this proceeding repeatedly. The same irritability was noted in the vacuoles lying among the ridges of the chloroplast, and the author claims to have seen small vacuoles arising from them, these passing forward in the cell and uniting with the terminal vacuole. The Brownian movement of granules is supplemented by another, slower, clearly visible up-and-down movement which was considered to be due to variations in pressure external to the vacuole causing changes in internal viscosity. To these changes the granules respond by a characteristic rolling, upward and downward movement. That the vacuole has a definite tonoplast is thought to be disproven by the fact that granules in their motion may pass out readily into the cytoplasm and return, an observation in agreement with that of Fischer. Wurtz concludes from his observations on *Cl. Dianae* and other species that there are three types of crystal-bearing vacuole; those with one crystal only, those with one large central granule and a mass of smaller ones, and those with elongated vacuoles containing a variable number of crystals. *Cl. didymotocum* has an indefinite number of crystals; *Cl. ulna, Cl. Archerianum, C. Silique* and others, but one; some species have two, one large and one small, and some species may have as many as thirty. Laporte (29) claims to have never seen a number of granules not easy to count, and according to his observations there are never more than ten. In any case it is logical to assume that gypsum granules are by-products of metabolism and that their number may vary between species and within the same species, according to differences in physiology.

CELL DIVISION

One would expect to find cell division to be unique among desmids, coordinate with their peculiar structure. In the unconstricted Saccoderm and the Gonatozygaceae, however, the process is not complicated, as it is in the true desmids. In the Mesotaeniaceae (*Netrium, Cylindrocystis*) the chloroplast elongates and then divides (the pyrenoid also), accompanying nuclear division. While this is in progress there appears in the median region an internal ring-like thickening of the wall. This develops centripetally, finally forming a cross partition which eventually splits apart to form the end walls of daughter cells, each containing two chloroplasts. Elongation of the new cells takes place in the apical region (68), whereas, on the contrary, in *Netrium* at least, observations indicate that growth occurs throughout the length of the cell. In *Cylindrocystis* cell division may be atypical of the desmids, occurring in two planes rather than one. In *Mesotaenium,* a simple type of cell, the single chloroplast is cleaved by the new septum. The separation of the two cells is achieved by gradual dissolution of the middle lamella in the septum. It is claimed that the break-down of the middle lamella contributes to the mucilaginous envelope.

In the Placoderm desmids cell division is accomplished in more than one way. Among the constricted genera (*Cosmarium, Euastrum, Micrasterias, e.g.*) the two semicells become moved apart by elongation of the isthmus, accompanied or followed by nuclear division. Then there appears within the wall at the mid-region of the elongated isthmus a cylindrical girdle band. From this wall-piece an inwardly growing projection begins to develop which eventually becomes a cross partition. At the same time the isthmus begins to constrict at this point and likewise to swell to initiate the new semicells. While this swelling is in progress the chloroplasts of the old semicells elongate and divide, thus contributing chloroplasts to the newly formed semicells. Finally, the cross partition, having been completely formed, splits to form the end walls of the new semicells which remain in juxtaposition until their walls are completely constructed. Often, however, the cells fail to separate, and short 'pseudofilaments' result (*Cosmarium moniliforme, e.g.*). In other cases (*Micrasterias foliacea, e.g.*) false filaments are formed by the interlocking of polar (apical) processes which in this case are hooked tips of apical lobules. When cell division is completed the sinus and isthmus of the two new cells are in the same relative position, namely, at the base of the old semicells with the two new semicells intervening.

When growth conditions are favorable cell division may be accelerated and occur before new semicells are completely formed. This, according to Playfair (39), accounts for variety of form and the "degenerate" expressions of species. He goes so far as to claim that whole groups of desmid species as described are but growth forms of the same species. He is of the opinion that ninety percent of described species of many types of organisms are "polymorphic forms". Such an analysis is open to question for a number of reasons and loses weight immediately when it is remembered that very often a new species is described without knowing whether the type is a true representative of the norm, or whether it is a 'variety'. To be sure, the nomenclature of desmids is heavily burdened with synonymy, much of which can be clarified only by frequent observations of collections from the same habitat or by culture studies in order to determine the range of variation which a species might show. Unfortunately desmids do not lend themselves easily to laboratory culture, although lately some advance has been made in devising suitable media. Czurda (10), for example, has grown *Mesotaenium* and *Cosmarium* on agar, finding that inorganic salts are sufficient for normal growth and cell division. Only *Mesotaenium* could use glucose and saccharose when organic compounds were employed as a primary medium. Similarly *Mesotaenium* could use glycogol and asparagin but not leucin, whereas *Spirogyra* used only asparagin. (See also 51 and 52 for notes on culturing Conjugatae).

As the new semicell wall develops it lays down structural features and patterns of decoration peculiar to the species—these showing a remarkable and almost unfailing duplication of those characteristics in the parent cell. So complex is the wall and so precisely are the complex patterns formed that the old question regarding the vitality of the cell wall comes to mind. Certainly the phenomena involved in the building of wall structures in desmids suggest a very close organic connection between the protoplast and its external envelope. It is inconceivable that an entirely lifeless cell wall could develop mucilage pores, scrobiculations and the many patterns of granules, spines and verrucae. Many such wall markings develop after the wall has been laid down, and they can not be credited to the activities of the protoplast alone. The new semicell wall is smooth and for a long time does not have the shape of the older cell half. Several days may be required to complete the new semicell wall. Lütkemuller (32) claims that the decorated mature wall of the new semicell develops internally to the first—formed smooth membrane which sloughs off. Other observations indicate that this is not true for all desmids, however, and Playfair (39) claims that wall decorations (*Pleurotaenium*) are the result of external conditions such as those which he ambiguously calls "stagnation".

Not infrequently the enlarging isthmus fails to follow through ordinary sequences. The cross septum may not be completed, and the swelling isthmus builds curiously shaped monstrosities between the two parent semicells. This condition is more frequently met with in *Euastrum, Cosmarium* and *Micrasteria* than in any other genera. Two or three abnormally shaped or partly reconstructed semicells may develop from the enlarged isthmus, growing out in two planes.

In *Penium* and especially *Closterium* cell division is complicated by the interpolation of new cylindrical wall sections. Preliminary to cell division the conical axial chloroplasts constrict and divide about half way between the midregion and the pole of the crescent-shaped cell. The nucleus undergoes mitosis and each daughter migrates to the position where chloroplast division has occurred. Then in a transverse plane just to one side of the midregion of the cell an internal thickening of the wall occurs. This develops at a point where the external layer of the wall is interrupted and where a faint line can be seen encircling the cell. In some species of *Closterium,* however, this interruption of the outer wall is a relatively wide break, and the girdle line is very prominent. The internal thickening develops therefore from an enlargement of the inner membrane in a line where the outer wall is lacking. The internal thickening next undergoes a curious lengthwise stretching and expanding, the result of which is the formation of a cylindrical section that lengthens the cell and forces the apical portions further apart. Then from the lateral walls of the new cylindrical piece a septum develops that divides the cell. The two daughters inherit each a section of the cylinder (girdle band) and an apical portion of the mother cell. From the new end walls, truncate at first but which begin to bulge and elongate, the apical portion of the new semicell takes its form. Thus, when fully developed, each daughter cell has an old semicell apex, a girdle band and a new semicell portion at the opposite end. Subsequent divisions result in cells with the original apical region at one end, a section of girdle inherited from the first division, a new girdle hand and an entirely new apical portion at the opposite pole. It is to be noted, however, that girdle bands do not form with every cell division, and in *Closterium,* at least, never more than two such bands are found, whereas there may be several in *Penium.* In some species girdle bands are never formed. The details of the girdle band type of cell division were first described by Lütkemuller (32).

An interesting interpretation of the manner in which the new semicell of *Closterium* is built was advanced by Wisselingh (77). His contention is that instead of the new end wall bulging and extending to form the new apex, another membrane develops internal to the first one that is laid down, and that it is this one which pushes out, as the daughter cells separate, to form the new horns of the cell. Hence the inner wall of the *Closterium* cell would be composed of a single piece, whereas the outer membrane would consist of several sections, old semicell portions and successively formed girdle band sections.

SEXUAL REPRODUCTION

Whereas a few cases of aplanospore formation are known among desmids (*Hyalotheca, Spondylosium,* possibly *Euastrum*), the universal method of reproduction, other than cell division, is by a sexual process. Asexual reproduction by zoospores, so common among most other members of the Chlorophyta and other algae groups as well, has been lost in the evolution of the Zygnematales from ancestral forms which probably employed zoospores. Sexual reproduction is accomplished by conjugation, a method of reproduction which is so fundamental that it would relate the Desmidiaceae and other desmids to the Zygnemataceae if the details of their cytology did not. Like other members of the Zygnematales, conjugation is carried on by non-flagellated aplanogametes, entire cell contents, or a major portion thereof, assuming the function of sex cells and moving in an amoeboid fashion.

The gametes from the two cells may be brought together either with or without interconnecting tubes, and there are many minor but interesting variations in the process of conjugation. In most of the observed cases in true desmids two individuals come into juxtaposition and are inclosed by a common mucilage. In taking up their position prior to conjugation, it is interesting to note, the angle of approach and contact varies according to the genus, the cells being parallel or inclined toward one another in various planes. The semicells separate and the contents migrate into the space between cells and fuse to form usually a single protoplasmic mass. Around this a zygospore wall of three layers is developed, the inner being thin and colorless, as in the Zygnematacae, the middle thicker, smooth, often brown and said to be cutinized, whereas the outer cellulose layer is frequently decorated with spines, warts or furcated hollow processes and arms. Especially in *Staurastrum* and *Micrasterias* this wall may be very ornate, but so precisely decorated that in many cases its markings may be used to identify species.

Genera regarded as more primitive among desmids (*Closterium*) have smooth-walled zygospores, whereas the more highly evolved forms are decorated. Such information as is at hand forces one to conclude that the conjugation process is less frequently employed in the more highly evolved genera. This is inconsistent, however, with the theory that filamentous desmids are evolved from unicellular forms, for *Hyalotheca* and *Desmidium* frequently are found in conjugation. This might be explained by the biology of one-celled and filamentous forms. Obviously filamentous species have more opportunity for the juxtaposition of physiologically different individuals than do solitary forms. But in *Cosmocladium*, where approximation of cells is facilitated by the common colonial mucilage, conjugation is not frequent. An obvious explanation of this might be that, inasmuch as a colony of these cells constitutes a clone, conjugation may not be possible, and that there is required the presence of physiologically different colonies.

Within the zygospore two chloroplasts, if four were involved in the fusion, disintegrate, whereas in plants containing but one chloroplast (*Mesotaenium*) one of the two chloroplasts breaks down. The gametic nuclei lie side by side during the dormant period, which may be over winter or for longer periods, and then fuse just before germination of the zygospore. There is considerable variation in the behavior of the germinating zygospore. Essentially, however, there are two successive divisions of the diploid nucleus, the first one being reductive, to form four haploid daughters. The content of the zygospore is liberated by a cracking of the thick wall, and once outside it constricts. Around the four nuclei as many embryonic desmid protoplasts may be organized. In other cases two cells are formed, each with two nuclei, and in each, one nucleus disintegrates so that two uninucleate cells result. Among the Saccoderm desmids (*Cylindrocystis*) division of the nucleus and of the protoplast may occur before the zygospore wall breaks down.

In life histories which have been critically studied (*Netrium*) it has been observed that the two nuclei in each new cell are of different sizes and that the smaller are the ones which break down.

In Gonatozygaceae only one new cell is formed from the zygospore, constituting one of the justifications for associating this family with the Zygnemataceae rather than with the desmids. There is considerable variation, however, among Saccoderm and Placoderm desmids, four new cells being formed in some species of *Mesotaenium* and but two in others, for example. There have been not altogether successful attempts to arrange desmids in an evolutionary series, according to the use that is made of the zygospore. Those forms (*Hyalotheca, e.g.*) in which three of the four nuclei disintegrate are regarded as more advanced, similar to the situation in the Zygnemataceae, whereas genera in which all four haploid nuclei give rise to cells are the primitive ones. Parthenospores of *Cosmarium* have been described (23) as producing but one new individual from four potential nuclei.

Another modification is found in the normal production of two functional zygospores in *Mesotaenium, Cylindrocystis* and some species of *Closterium.* The twinning results from the fact that each cell divides prior to conjugation, thus producing four gametic protoplasts.

In the Mesotaeniaceae, and apparently in most of the Desmidiaceae, a conjugation tube, often very thin and evanescent, brings the gametes together. This develops either after (*Hyalotheca*) or before the cells have come in contact. In most members of the former family broad papillae extend toward one another, meet and fuse. In the rare genus *Roya* a specialized variation occurs in the appearance of exit pores in the ends of the unconnected papillae through which the gametes pass out and fuse. The zygospores are formed between the two cells, which use tubes, except in *Desmidium cylindricum* where the cells of one filament (female) receive the gametes from the mating (male) plant, and in *Hyalotheca* (22). Another possible indication of an incipient anisogamy is seen in *Closterium parvulum* where the contents of one cell are more active and pass into the conjugating tube well ahead of the other (female) gamete.

In most of the filamentous genera the cells dissociate before conjugating, but in *Desmidium cylindricum,* although dissociation has been observed, and in a few others (*Gymnozyga, e.g.*) there is scalariform conjugation, as in the Zygnemataceae. In rare cases conjugation is lateral (*Desmidium* spp.).

Although actual hybridization has been observed rarely (3), many examples well known to the author, and others referred to in the literature, suggest that it may occur frequently. Apparent hybrids are found especially in *Micrasterias* and *Euastrum* where the two mature semicells bear pronounced characteristics belonging to two different species.

EVOLUTION AND TAXONOMIC POSITION

The origin of the Zygnematales is obscure, and the phylogenetic relationships of the desmid families to the group as a whole are subject to much speculation. At least the order is now recognized by most phycologists to be of good standing as a component of the Chlorophyta and is no longer segregated, as has been done previously (53, 76). Pigmentation and the nature of assimilatory products are fundamental characteristics and are among the most important ones to be considered in either grouping or separating plants, especially phyla of algae. But whether the group evolved from a filamentous ancestor of the Ulotrichine line or came up directly from a unicellular progenitor in the Volvocales is not clear. Nor can solutions be found unless interrelating forms be discovered to support one of the two views. Fossil evidence is entirely lacking to date. The method of reproduction, the lack of motile cells (vegetative and reproductive), the almost universal occurrence of axial chloroplasts, in combination with certain other cytological pecularities would seem to preclude desmids as derivatives of any filamentous Chlorophyta known today. At the same time certain of the characteristics, especially lack of motility and retention of the ability to reproduce vegetatively by cell division, make it difficult to relate the Zygnematales to either the Volvocales or the Chlorococcales (57 to the contrary). Stress has been laid on the unusual habit of certain Volvocales to use conjugation and amoeboid gametes (*Chlamydomonas eugametos, e.g.*) as a clue to the ancestry of the Zygnematales. In addition, the fact that the form and position of the chloroplast in the Volvocales, although predominantly parietal, is varied makes it not unreasonable to assume a possible origin from that order. At present this seems to be the most acceptable view. In any case the Zygnematales have come far in their evolution and now occupy an isolated position; like the Characeae, phylogenetically they have no known ancestors nor progeny.

The various ideas concerning phylogeny of the group resolve themselves into two notions regarding the historical relation of desmids to the rest of the Zygnematales. One is that the unicellular forms have come about by a dissociation of filamentous ancestors; the other, that the filamentous Zygnematales are derived from unicellular desmid genera. The latter view is very reasonable and has some consistency with the theory that the origin of the Zygnematales lies among the unicellular Volvocales. Thus one can envisage a *Chlamydomonas* type of cell with a conjugation method of reproduction giving rise to a primitive unicellular desmid. From this there may have diverged two or more lines of development leading to modern Zygnematales. One line would have resulted in the Gonatozygaceae-Zygnemataceae; another, the higher Desmidiaceae. In the latter a filamentous expression would culminate the series in the genera *Hyalotheca, Desmidium, Phymatodocis, etc.* Many phycologists hold to this view (34, *e.g.*).

It might be contended that such a sequence is untenable because vegetative complexity (morphology of *Staurastrum* and *Micrasterias*) is not necessarily accompanied by a higher status in respect to zygospore germination. That is, some simpler vegetative cells have a single new individual formed from a zygospore, a behavior which is true, on the other hand, for the filamentous (more highly evolved) *Hyalotheca* (40). It is well known, however, that throughout evolution in all groups of organisms there may be greater development in one set of characters than in others, even among closely related species. Numerous examples occur in the algae themselves (heterogamy in the simple colonial forms such as *Pleodorina, e.g.*).

To be considered in the evolution of filamentous desmids is the fact that their cell architecture is simple, indicating that they have had unspecialized immediate ancestors. Also their frequent sexual reproduction, already referred to, is interpreted as being a primitive characteristic and one which is common among the forms less advanced vegetatively.

West (68), in reviewing phylogenetic theories, dismisses the notion that unicellular desmids could be regarded as ancestors of filamentous Zygnematales because he interprets available evidence to indicate that they have sprung from filamentous forms, and boldly affirms: "The average Desmid must be regarded as a unit of a dismembered filament". West does not seem to recall, however, that it is not necessarily claimed by the advocates of the other theory that the modern highly specialized desmid unicells, as such, are ancestors, but that the simpler forms or their immediate ancestors very likely might have given rise to the higher filamentous Zygnemataceae. The author contends that there is just as strong evidence, inferential and otherwise, that filamentous desmids and higher Zygnemataceae evolved from unicellular forms, as there is for the converse. West and others use *Debarya desmidioides* as a case in point. In this filamentous member of the Zygnemataceae there is dissociation prior to conjugation, and solitary cells put out conjugation tubes, thus strongly suggesting a degeneration to a unicellular desmid form. The ease with which some species of *Mougeotia* dissociate in vegetative condition is another bit of evidence which one might interpret as a reversion to a one-celled ancestral type as easily as one might assume such a case to be an upward evolution toward unicellular desmids. In his argument West fails to use the very frequent tendency of unicellular forms (*Cosmarium, Pleurotaenium, Micrasterias*) to form filaments as possible evidence that there has been an evolution in that direction. Rather he regards this as an entirely incidental characteristic assumed by unicells which themselves have had filamentous ancestors.

Much light can be thrown on the question of desmid evolution by considering the interesting trends which apparently have been followed in the development toward complex forms (*Micrasterias, Staurastrum*). These trends have been clearly elucidated by Fritsch (16). When the array of morphological types is reviewed it seems probable that the primitive cell was cylindrical, radially symmetrical (circular in cross section) with truncate or slightly rounded poles, and not constricted in the midregion. Such a cell type is found in *Cylindrocystis, Mesotaenium* and *Genicularia*. Constrictions of such a cell, slight at first, would yield such forms as are found in *Penium*. It appears likely that with constriction came a rounding of the apices to give the *Cosmarium-Arthrodesmus* expression, at first simple and smooth walled. From such an architecture compression of the cells occurred so that instead of being round in vertical view they became elliptic or had parallel margins. From this type of cell lines of development can be traced to other more elaborate genera (*Euastrum, Micrasterias*), and that these trends must have occurred is substantiated by the fact that there are many intergrading expressions. Frequently it is not easy to assign a plant to a genus inasmuch as it possesses a combination of generic characteristics (*Staurastrum polonicum, Euastridium verrucosum*). As Fritsch (16) points out, one tendency is for the simple *Cosmarium* type to develop the pyramidal-shaped cell (*C. pyramidatum, C. granatum*) accompanied by a deepening of the constriction until a narrow closed sinus results. Another modification is seen in the elaboration of the cell wall, at first plain, then with simple, uniform decorations, finally developing elaborate patterns and heteromorphic ornamentations in the more advanced forms. Following the development of the flattened cell, to which reference was made above, further modification has produced cells with facial swellings which in vertical view show various degrees of prominence. At first these were only thickenings of the wall (*C. refringens*), later decided smooth protuberances, and as a climax became prominent swellings with localized and complicated ornamentations. Extention of the *Cosmarium* cell in another plane is thought to have produced the ancestral type of the genus *Staurastrum*. In that genus, however, triangular cells in vertical view predominate, whereas the modified *Cosmarium* type of cell normally would be expected to have evolved quadrangular *Staurastrum*. There are enough instances of triangular or nearly triangular *Cosmarium* species or varieties to support the theory that tri-radiate *Staurastrum* species have evolved from them. It is noteworthy that the tri-radiate *Staurastrum* species are more *Cosmarium*-like in their ornamentation and in their simplicity of form than are the four-, five-, six- and eight-radiate species. The same tendency to form tri-radiate cells is seen in *Xanthidium* and in the varieties of some *Micrasterias* species (*M. Murrayi* var. *triqueter*). If radiate architecture is evidence of an evolutionary trend, then *Staurastrum* is the present culmination. The many hundreds of species with their innumerable shapes make it easy to postulate that evolution from more than one ancestral type has occurred to produce the complex genus. The several facies which appear in the genus have led some students to divide it, and they have at different times used names which have fallen into synonymy (*Didymocladon* Ralfs, *Pleurenterium* (Lund.) Wille, *Dichotomum* West & West, *Euastridium* West), although the latter name is retained by some students. Teiling (64*a*) has segregated monospinous species of

Staurastrum, grouping them with certain triradiate *Arthrodesmus* entities to form a new genus, *Staurodesmus.*

Finally another significant trend has been the appearance of the parietal chloroplast which, according to some, has come about by the separation of radial ridges of the primitive and characteristic axial type. This presupposes that the axial core has undergone a degeneration, and means that *Pleurotaenium, Xanthidium* spp. and some forms of *Cosmarium* are highly evolved in this respect, even though they are not so advanced along some of the trends. In general, however, the several tendencies exhibited in evolution within the desmids are seen to have occurred as parallel developments.

OCCURRENCE OF DESMIDS

It is in ecology and distribution that desmids show some interesting phenomena. Although some variation in habitat is permitted them, they are to be found mostly in acid (pH 4.8–7.0) and soft water situations. In general, the Saccoderm desmids (*Cylindrocystis, Netrium*) are less restricted than are the true desmids, inasmuch as the former occur in a wider pH range and may be found in hard water or on basic soils. Some species of *Closterium* and a few planktonic forms of *Staurastrum* commonly appear in habitats with a pH above neutral. *Sphagnum* bog pools are invariably desmid haunts, especially in temperate zones of the northern hemisphere, and in such associations they show the greatest production in numbers of individuals. Here they occur in gelatinous masses, free-floating in shallow warm water, or adhering to submerged aquatics, sometimes forming bright green films over mosses, or as periphyton on dead rush stems. Even if not visible their presence can be detected by the slippery quality imparted to wet *Sphagnum,* and a favorable method of collecting is by squeezing water from the moss through a bolting cloth net. Non-calcareous lakes overlying silicious rock, or pools fed by streams through igneous outcroppings are also favorable habitats, particularly in the north temperate zone and in alpine and subalpine regions. In the latter situations their bulk may not be great, but the number of species is large. This is true also for shallow pockets and ditches in sand dunes and sand plains in North America (New Jersey, Florida, Louisiana). Rich desmid floras, in both quantity and quality, develop in shallow poorly aerated water where there is a concentration of partly decomposed organic matter and where there is an abundance of organic acids. Shallow Pudding Pond in New Hampshire, well known to early desmidologists in America, is a veritable jelly mass because of the tremendous development of both species and individuals. This pond is surrounded by a land flora of acidophilic plants (*Drosera, etc.*).

Although a great majority of desmids are strictly aquatic (always fresh-water), some are semi-terrestrial (subaerial) and are to be found growing among bryophytes on soil and on dripping rocks (*Cylindrocystis, Closterium* spp., especially). A limited number of species (in *Staurastrum, Xanthidium,* and a few in *Micrasterias*) are well adapted to a planktonic existence by virtue of increased buoyancy provided by radiating hollow arms and spines, with some assistance from their gelatinous sheath. Occasionally, and perhaps only incidentally, some species are found in streams. The rare genus *Oocardium* (previously mentioned) is found in calcareous encrustments usually on stones in flowing water. Desmids occur at all altitudes where water chemistry is suitable, and are known from practically all latitudes from which collections have been made.

FACTORS GOVERNING THE DISTRIBUTION OF DESMIDS

There has been enough time for desmids to have attained a wider and more general distribution than most of them have if it were not for limiting factors. This is obviously true for most organisms, but is sharply illustrated by the desmids because of their fine sensitivity to environmental conditions. This is evidenced by the worldwide and common occurrence of some species in particular kinds of habitats only, and by the rare occurrence of other species in stations remote from one another. Thus, for example, *Closterium Ehrenbergii* is generally distributed (almost ubiquitous), whereas an East-Indian and African species, *Pleurotaenium ovatum,* is known as a rarity from Panama and Florida. *Euastrum pseudopectinatum,* reported from Africa, was next found in northern Michigan; and *Staurastrum polonicum,* known only from central Europe for a long time, has been found in the mountains of western North America (43). *Euastrum informe* was described from Brazil and next made an appearance in small ponds on Cape Cod, and *Spinoclosterium curvatum,* described by Bernard from Java, has since been found in a number of places in temperate North

America (45, 75). *Micrasterias foliacea,* a beautiful filamentous species, and *Phymatodocis Nordstedtiana,* often referred to in literature as tropical, are known from many northern stations (Maine, Michigan, Canada). At the same time there are blocks of species which seem to maintain continental segregation. Hence, to a limited degree, reference can be made to African, American, Arctic, western Europe, and Malayan types of desmid floras.

Taxonomic lists of desmids reported from many parts of the world and from different types of habitats have accumulated to the extent that concepts of their distribution are beginning to clarify. The picture is still hazy, and lines are not well drawn partly because the literature is so bulky that summarizing analyses are difficult. Whereas many species seem to be characteristic of geographical areas and indeed may be classed as endemic, generalizations are continually being broken down as information increases and as supposed endemics are reported from far away stations.

The alpine flora, better understood in Europe than elsewhere, shows continental variation and cannot as yet be generalized on. The high altitude desmids in Colombia (64, 67), for example, present an aspect different from the Andean flora of Ecuador just to the south, according to limited information from the author's collections, and these in turn are strikingly varied in composition from the known alpine associations in western North America (8, 43, 62).

Tropical desmid floras are made up of a complex in which endemics are conspicuous, even dominating in some collections. Brazilian and Indo-Malayan desmids, better known than those from other tropical regions, show many unique features (4, 18, 22, 33, 65). Even so, the Brazilian flora is found to have interesting similarities throughout Central America (44) and the Gulf region of the United States (48–50). In respect to tropical distribution desmids, and perhaps Zygnematales as a class, are in contrast to some other orders of the Chlorophyta such as the Volvocales and Chlorococcales, for in the latter there seem to be no members definitely confined to the equatorial belt. Genera which contain tropical endemics are principally *Micrasterias, Staurastrum* and *Euastrum;* there are also a few species of *Pleurotaenium* and some filamentous forms.

What are the conditions which determine similarities and differences in desmid floras in different areas, and what are the impositions which make for desmid habitats? Interconnecting bodies of water and continental geography of the remote past have assisted in distribution, of course. Chance scattering of zygospores by wind and water fowl may account in part, and chance collecting has influenced our ideas of desmid distribution. But beyond this it is apparent that particularly suitable ecological conditions are involved and that one reason that more desmid species are not ubiquitous is some peculiar physiological demand. A satisfactory understanding of desmid ecology certainly involves their physiology, about which little is known so far in spite of the many attempts to culture them (30, 35, *e.g.*).

Much is known about the relationships of desmids to water chemistry without knowing precisely what the all-important determining elements or substances are. It was early recognized by phycologists that desmids thrive in acid situations and that they are calciphobic. West and West (71, 72), studying desmid distribution in the British Isles, advanced the idea that bodies of water lying in Precambrian rock (hence very ancient bodies of water) were the habitats which made for a rich desmid flora (200–300 species) and a phytoplankton predominantly of desmids. An association with old Paleozoic rock was noted especially in their studies of Scottish lakes which have a rich desmid flora. The Capel Curig lakes in Wales are the richest in the world, the Wests claim (72). These authors did not agree, at least at first, with the contention of other observers (Wesenberg-Lund, *e.g.*) that peaty water, regardless of its geological age, was the answer to desmid distribution. They claimed that the paucity of desmids in Switzerland is explained by the geological formations being "too recent". The Wests found ample evidence in England, however, that lakes of the Carboniferous and younger were poor in desmids, even though peaty water drained into them. They found an interesting correlation between abundance of desmids, rainfall and Archean rock formations in the British Isles which they show in a map (72). Furthermore the Wests contend that the great age of a body of water produces a larger number of species in a habitat (200–300), whereas the lack of calcium determines a larger number of individuals. Without reporting any water analyses the Wests (73) agree that the chief factor determining the quality of desmid floras is a chemical one. Wesenberg-Lund (66) has remarked on the well known fact that glaciation in many areas obliterated most of the characteristics belonging to an ancient terrain of igneous rock, which according to Wests precisely determines a desmid flora.

An analysis of water which supports a desmid flora shows many significant correlations with water chemistry. The Caledonian type of lake, favorable for desmids, is low in nitrogen and phosphorus, low in calcium and magnesium, but often relatively high in sodium and potassium. The Baltic lake is calcareous, often high in nitrogen and phosphorus, and has a correspondingly high pH (pH 7.2–9.8). The observations of European phycologists in desmid distribution are borne out in part by the studies of desmid floras in North America (21, 42–50, 58, 63, 80). As Smith (58) has pointed out in his studies of the Wisconsin flora, Caledonian types of lakes in northern Wisconsin support relatively rich desmid floras, whereas the Baltic lakes in the southern part of the State, overlying and drained from calcareous soils, are poor in these plants but rich in Cyanophyta-Diatom species. Lakes in the two areas are approximately of the same age, however, and it is clearly a matter of chemical constitution of the water and not the age which determines the desmid flora. The present author has found some lakes in northern Wisconsin to be slightly basic and relatively poor in desmids (Shell Lake), whereas a few lakes in southern Wisconsin in the limestone area are moderately soft and support a relatively conspicuous desmid flora, occasionally rich in shallow water of marshy borders where there is a concentration of organic acids. Such lakes are affected by geographical and geological factors in such a way that the mineral content is not high, and the pH is low (pH 6.8–7.0–7.2). Ditches and small pools in Louisiana, relatively recent in formation and some not even permanent bodies of water, are highly acid (pH 6.2–6.8) and are luxuriant in number of species. Joshua (22) reports an interesting list of desmids, including many new species, from an ancient small tank near Rangoon which, because of its never having been disturbed for many years, had apparently built up a high organic acid content. The region is composed of an alum-producing sandstone. At Vincent Lake in northern Michigan, a retrograding bog lake, a rapid drop in water left a barren sand beach. Within four years the beach became invaded by *Sphagnum* spp., *Drosera rotundifolia, Lycopodium inundatum,* and in the carpet where small depressions were formed there now occurs an extremely rich and varied desmid flora. Whereas the lake has a pH of 7.1 (summer), the pools in the surrounding mat are 6.6. Such records contradict the theory of the Wests that bodies of water must drain ancient rock in order to be suitable for desmids.

As Pearsall[5] has pointed out, phytoplankton of "primitive" lakes in which the sodium-potassium/calcium-magnesium ratio is high are characterized by a desmid flora, whereas a Cyanophyta-Diatom flora predominates when the ratio is low. Rocky lakes (oligotrophic type) may have a ratio of 3.2, and silted (eutrophic) lakes a ratio of 1.1. An example of the numerous studies bearing on the Caledonian-desmid relationship is Messikommer's recent work. In the mountain region of St. Galler Oberland near Grauen Hörner the algae of several lakes were studied. The habitats ranged in altitude from 485 meters to 2,441 meters. The lower ones (485–930 meters) were relatively high in pH (7.4–7.5) and had an alkalinity of 11.0–12.5. These were predominantly diatomaceous (246 species) with several blue-green species, whereas lakes at 1,902–2,441 meters were mostly soft water (alkalinity 2.0–10.0) with a pH ranging from 6.55 to 7.0 (7.4 in one case) and had a conspicuous desmid flora (104 species). It is significant that there was no temperature correlation.

In Denmark and northern Germany water is calcareous and comparatively poor in desmids, whereas in Scandinavia, Finland and Switzerland there is a high Na-K/Ca-Mg ratio. Desmid floras in these areas, especially in Denmark, are abundant, showing, incidentally, a great similarity to the flora of the glaciated regions of eastern United States and Canada. The Scottish desmid flora also has a composition like that of northern Europe and eastern North America, the so-called "western" flora (70).

It is now rather generally agreed that the early notions of the Wests are correct in so far as water overlying igneous rock is favorable for desmids. Such rock formations are hard, and water draining through them carries few minerals in solution. The causal relationships, however, lie in the fact that such lakes are low in calcium and have a pH below neutral. Whether calcium itself restricts desmids or whether the presence of optimum amounts of nutrients (organic acids) found only in acid or soft water situations makes their growth possible has not been determined. The question of the antagonism of ions and the likeliness of growth-promoting substances being present come to mind. Certainly fine physiochemical balances are struck and there is every indication that chemical substances as yet undetermined control the presence and absence of desmids in a body of water. The pH alone cannot be considered a decisive factor but rather should be regarded as an index of a chemical condition suitable or unsuitable for desmids.

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[5] See Pearsall for an interesting table comparing bodies of water from over the world in respect to their hardness, their Na-K/Ca-Mg ratio and correlated types of plankton.

Temperature as a factor in desmid ecology appears to be important in the determination of numbers of individuals in a habitat but appears inconsequential in distribution. In any case, many species known from the tropics are also found in subtropical and alpine habitats. Although there is an ill-defined tropical flora it is not temperature alone that is concerned in its determination, or at least only indirectly so. Vegetative activity and cell division are accelerated in warm water, however, which in turn provides for greater zygospore formation simply because there is more opportunity for physiologically different cells to become apposed. As mentioned previously, very cold water trickling from glaciers and snow fields, conditions other than temperature also being favorable, may support a flora rich in desmid species. In their plankton studies West and West (74) have found that desmids are most abundant in the autumn just after the peak of summer temperatures. Their observation, while having some significance, cannot be used as a basis for generalization in view of evidence to the contrary contributed from ecological studies in other countries.

In the tropics high temperatures reduce the ability of water to retain gases and the low CO_2 content is thought to be related to the fact that desmids near the equator tend to form filaments. At least filamentous genera are very abundant. It is well known that desmids as a class thrive in poorly aerated waters. Myxophyceae also are familiar inhabitors of such situations, but with these plants there is a demand for nitrogen, phosphorus and calcium bicarbonate, substances not conducive to desmids. It is interesting to note that a few blue-greens can thrive intermingled with desmids in poorly aerated water. These are mostly *Stigonema ocellatum, Hapalosiphon pumilus, Chroococcus turgius, C. Prescottii* and *Aphanocapsa* spp.

The selectivity of desmids and factors which determine their distribution have created what appear to be consocies of species. These associations have been more fully studied in Europe than elsewhere (1, 11, 29, 38). LaPorte (29) has painstakenly summarized taxonomic lists of European desmids and has been able to establish ten types of associations. These are significant even though the lack of similar studies in other countries prevents a general application at present. Students of the desmids on other continents can recognize similar associations in their individual researches, however.

There is first the Aerial Association (29) where humidity makes it possible for cells to exist on soil and trees. *Mesotaenium* spp. are known from such habitats.

The Subaerial Association is known as Desmidiaceaetum aerophilum wherein a few forms of *Cosmarium* (*C. microsphinctum, e.g.*), *Cylindrocystis crassa* and *Tetmemorus minuta* live on moist acid soil around *Sphagnum* cushions in alpine regions near melting snow. The author has found such an association in Cranberry Lake, a small marshy area at a low altitude in the Cascade Mountains of Oregon.

A third association is the Closterietum commune, dominated by *Closterium* spp. but accompanied by many diatoms and a few *Cosmarium spp.* Newly formed ditches, especially when layered with dead leaves, and trickles of water on banks are habitats of this association. Exactly similar situations were found by the author on dripping banks beside mountain trails in Ecuador. Such habitats are obviously basic rather than acid and naturally would have only a few selected species which, however, tend to be ubiquitous because of their greater range of water chemistry tolerance, indicated by a wide pH range.

The Cosmarietum transitorium has a much larger association of species—a mixture of forms occurring both in the former group and in the following Euastro-Micrasterietum. Habitats for this consocie are mossy bogs which, although slightly acid, support a conspicuous diatom flora. *Cosmarium* spp. (*C. anceps, C. Boeckii, C. granatum, et al.*) predominate, however, with occasional *Euastrum* spp.

The fifth association (Euastreto-Micrasterietum) is a complex of eight genera of desmids inhabiting *Sphagnum* bogs along with

Carex rostrata and *C. fusca* in water with a low pH (pH 5.0–6.0). Here larger numbers of individuals occur and a definite desmid habitat appearance is given to the flora. Because the water level may fluctuate, however, the composition may change radically throughout the season; in drying and in the concentrating of inhibiting substances, the Euastrum-Micrasterietum complex may be superseded by *Closterium* spp., followed by a Euglenoid flora, the sequence being reversed with the diluting action of returning rains.

The Closterieto-lineati-Pinnularietum-stauropterae, not well defined, is based principally on Messikommer's list of 200 species of algae from Swiss marshes dominated by *Carex rostrata* and *C. limosa*. *Cosmarium* spp. are conspicuous but there are many diatoms. As LaPorte points out, it seems to be an association which has the same relation to other bog floras that benthic desmid consocies have in strictly aquatic habitats.

The seventh association is known as the Cosmarieto-Staurastretum and is composed of plants free-floating or adhering to *Utricularia* and other aquatics in soft water habitats. Here is found a mixture with *Cosmarium* and *Staurastrum* spp. dominant, usually in the deep water of bog lakes.

The Desmidiaceatum-benticum is also ill-defined, as previously indicated, and includes numerous species of all genera which, according to the author's experience, are as likely to be found in the shallow water of bogs as in the benthon. It is doubtful that such an association actually exists *per se*.

On the contrary the planktonic association (Micrasterias-Staurastretum planctonicum) seems to be more definite and includes a group of species which nearly always are found in open water. This is especially true for *Staurastrum* spp. (*St. tetracerum, St. Cerastes, e.g.*) in North American lakes, and *Micrasterias radiata* and *M. americana*.

Lastly are the calciphilic desmids which constitute an association that might well grow out of the Cosmarietum transitorium. It is composed mostly of *Cosmarium* and *Closterium* spp. which show adaptability to mineralized water.

In respect to the dynamics of these associations LaPorte suggests a transition from Closterietum commune to Cosmarietum transitorium to Euastreto-Micrasterietum. From the latter there is a trend on the one hand to Desmidiaceatum aerophilum and on the other to Cosmarieto-Staurastrum and then to the Closterieto-lineati-Pinnularietum-stauropterae. Such transitions are in keeping with the hydro-geographic evolution of lakes and bogs which finally become unsuitable for desmids.

Further analyses of desmid ecology and the application of such generalizations as suggested by LaPorte are needed. Year-around collections from selected habitats such as the careful work of Irenée-Marie in Canada, especially if accompanied by chemical analyses, will eventually answer many of the interesting ecological questions. As Fritsch (16) points out, studies of desmid dynamics will yield necessary and helpful information more than the popular taxonomic investigations, important though the latter may be.

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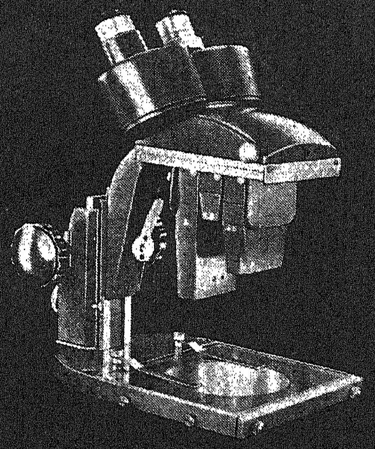

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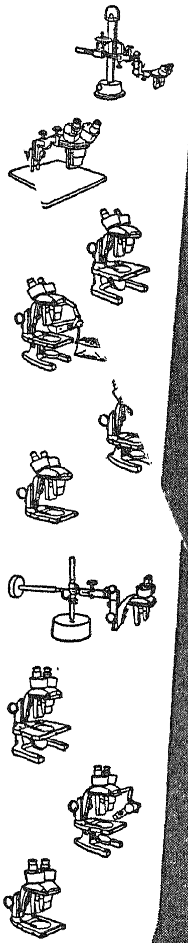

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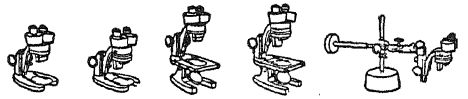

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